from ligroin (bp 60–80 °C) to afford pure 33 (0.063 g), mp 83–85 °C.

(*Z*)-α-Methyl-*m*-(trifluoromethyl)cinnamic Acid. A solution of 14 (2 g) in EtOH (250 mL) was irradiated for 60 h as described above to afford a residue whose NMR spectrum showed, in addition to the signal at δ 7.95 of the vinly proton of 14, a signal at δ 6.92 attributable to the vinyl proton of the (*Z*)-acid. The relative areas of these two signals showed the mixture to consist of the *Z* and *E* isomers in a ratio of about 36:65. However, all attempts to obtain pure (*Z*)-acid from this mixture were unsuccessful.

Pharmacology. Procedures for measuring the anticonvulsant activity and behavioral effects have been previously described.[2] Icem-CET (SPF Caw) male albino mice, weighing 17–22 g, fasted for 9 h, were used. The test compounds were suspended in 0.5% methocel (90 C HG 400 cP) and administered by gavage in a volume of 0.2 mL/10 g of body weight, using suspensions at different concentrations. The ED_{50} values for pentylenetetrazole antagonism were calculated by probit analysis,[25] carried out on the results obtained from groups of 20 animals per dose level (four or five doses for each product); animals were given the compounds 30 min before the pentylenetetrazole (130 mg/kg ip).

Acknowledgment. This work was supported in part by a grant from Consiglio Nazionale delle Ricerche (Roma).

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Structure-Activity Relationships in a Broad-Spectrum Anthelmintic Series. Acid Chloride Phenylhydrazones. 1. Aryl Substitutions and Chloride Variations

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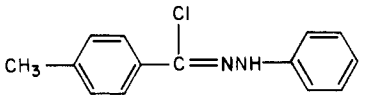
The discovery of the broad-spectrum anthelmintic candidate *p*-toluoyl chloride phenylhydrazone was first reported in 1973. This account describes changes in anthelmintic activity with variations in substituents on the phenyl rings and modifications of the chloride moiety. Several congeners in the acid chloride phenylhydrazone series have efficacy against a variety of helminths of domestic animals.

The currently marketed broad-spectrum veterinary anthelmintics include a profusion of benzimidazoles and tetramisole.[1] Benzimidazole-resistant strains of *Haemonchus contortus*, *Trichostrongylus colubriformis*, and *Ostertagia circumcinta* have appeared.[2] The development of resistance makes discovery of an anthelmintic with a novel biochemical–pharmacological mode of action a high priority for the medicinal chemist with a veterinary helminthology orientation. Our screening and lead evaluation program resulted in the discovery of *p*-toluoyl chloride phenylhydrazone (**1a**), a compound with outstanding ef-



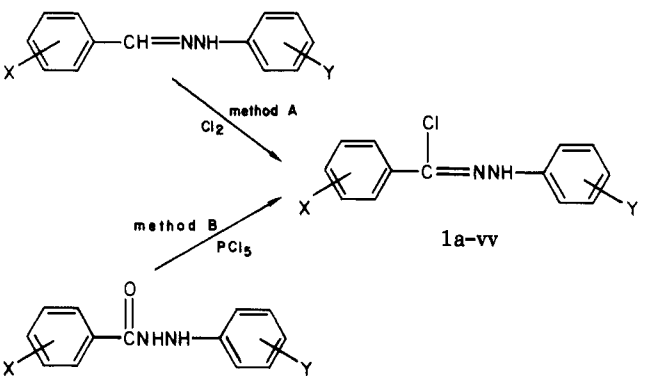
1a

ficacy against common nematode and cestode infections in sheep.[3] The chemical structure of **1a** represents a radical departure from previously known anthelmintics. therefore, **1a** is potentially a prototype of a class of compounds with a novel mode of action. The present paper describes modifications of the *p*-toluoyl chloride phenylhydrazone structure and resultant effects on anthelmintic activity in laboratory and domestic animals.

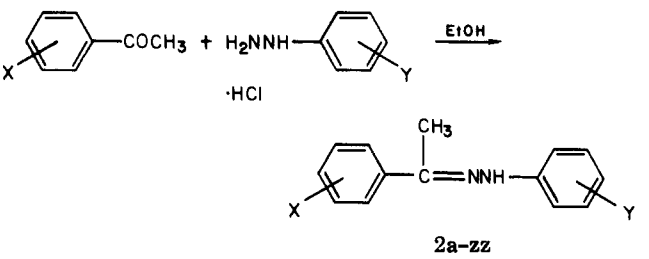
Results

Chemistry. Synthesis of the benzoyl chloride phenylhydrazone **1** was accomplished by either chlorinating the benzaldehyde phenylhydrazone (Scheme I, method A; see preparation of **1ss** as an example) or reacting the benzhydrazide with phosphorus pentachloride (method B, see preparation of **1a** as an example). The chemistry and characterization data for series **1** have been reported.[4] The

Scheme I



Scheme II



acetophenone phenylhydrazones **2a–zz** were formed by reacting equivalents of the acetophenone and phenylhydrazine under conventional conditions (Scheme II). Chemical and physical characterization data for **2a–zz** are found in Table I. Unfortunately, many compounds in this series decompose to dark tars on standing at room temperature or upon attempting to vacuum dry. Structures are assigned on the basis of known chemistry and spectra. The benzophenone phenylhydrazones **3a–f** were prepared by essentially the same methods used to form **2**. The

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(2) J. D. Kelly and C. A. Hall, *Adv. Pharmacol. Chemother.*, **16**, 89 (1979).
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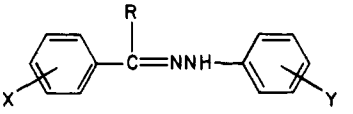
0022-2623/81/1824-0532$01.25/0

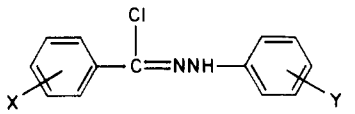
Table I. Aryl Ketone Phenylhydrazones



no.	X	R	Y	mp, °C	% yield	crystn solvent	formula[a]
2a	4-CH_3	CH_3	2,4-Cl_2	123.4	72	EtOH	$C_{15}H_{14}Cl_2N_2$ [b]
2b	4-CH_3	CH_3	2,3-Cl_2	116.0	83	EtOH/H_2O	$C_{15}H_{14}Cl_2N_2$ [c]
2c	4-CH_3	CH_3	2,5-Cl_2	117.0	84	EtOH/H_2O	$C_{15}H_{14}Cl_2N_2$ [d]
2d	3-NO_2	CH_3	H	127.7[g]	82	EtOH/H_2O	$C_{14}H_{13}N_3O_2$
2e	3-NO_2	CH_3	2,4,6-Cl_3	155.4[h]	69	EtOH/DMF/MeOH	$C_{14}H_{10}Cl_3N_3O_2$
2f	3-NO_2	CH_3	2,3-Cl_2	168.4	81	EtOH/DMF	$C_{14}H_{11}Cl_2N_3O_2$
2g	3-NO_2	CH_3	2,4-Cl_2	138.4	94	EtOH/DMF	$C_{14}H_{11}Cl_2N_3O_2$
2h	3-NO_2	CH_3	2,6-Cl_2	124.3	92	EtOH/DMF/H_2O	$C_{14}H_{11}Cl_2N_3O_2$
2i	3-NO_2	CH_3	3,5-Cl_2	194.1	87	EtOH/DMF/H_2O	$C_{14}H_{11}Cl_2N_3O_2$
2j	3-NO_2	CH_3	4-CH_3, 3-Cl	133.0	91	EtOH/H_2O	$C_{15}H_{14}ClN_3O_2$ [e]
2k	3-NO_2	CH_3	2-CH_3, 3-Cl	158.0	71	EtOH/DMF/H_2O	$C_{15}H_{14}ClN_3O_2$
2l	3-NO_2	CH_3	2-CH_3, 4-Cl	159.1	86	EtOH/DMF/H_2O	$C_{15}H_{14}ClN_3O_2$
2m	3-NO_2	CH_3	4-Br	156.7	93	EtOH	$C_{14}H_{12}BrN_3O_2$ [f]
2n	2,4-$(CH_3O)_2$	CH_3	4-Br	127.7	25	EtOH	$C_{16}H_{17}BrN_2O_2$
2o	2,4-$(CH_3O)_2$	CH_3	4-CH_3	142.1	33	EtOH	$C_{17}H_{20}N_2O_2$
2p	2,4-$(CH_3O)_2$	CH_3	2,4-Cl_2	112.6	41	EtOH	$C_{16}H_{16}Cl_2N_2O_2$ [i]
2q	2,4-$(CH_3O)_2$	CH_3	2,3-Cl_2	129.5	53	EtOH	$C_{16}H_{16}Cl_2N_2O_2$
2r	2,4-$(CH_3O)_2$	CH_3	3,5-Cl_2	90.6	86	EtOH	$C_{16}H_{16}Cl_2N_2O_2$
2s	2,4-$(CH_3O)_2$	CH_3	2,6-Cl_2	109.7	38	EtOH	$C_{16}H_{16}Cl_2N_2O_2$ [j]
2t	2,4-$(CH_3O)_2$	CH_3	2,4,6-Cl_3	112.9	67	EtOH	$C_{16}H_{15}Cl_3N_2O_2$
2u	2-OH, 4,5-$(CH_3)_2$	CH_3	2,4-Cl_2	184.4	80	EtOH	$C_{16}H_{16}Cl_2N_2O$
2v	2-OH, 4,5-$(CH_3)_2$	CH_3	3,5-Cl_2	189.2	76	EtOH	$C_{16}H_{16}Cl_2N_2O$
2w	2-OH, 4,5-$(CH_3)_2$	CH_3	2,6-Cl_2	120.8	69	EtOH	$C_{16}H_{16}Cl_2N_2O$
3a	4-CH_3O	4-$CH_3OC_6H_4$	2,4-Cl_2	161.7	70	EtOH/DMF	$C_{21}H_{18}Cl_2N_2O_2$ [k]
3b	4-CH_3O	4-$CH_3OC_6H_4$	2,3-Cl_2	114.4	60	EtOH	$C_{21}H_{18}Cl_2N_2O_2$
3c	4-CH_3O	4-$CH_3OC_6H_4$	3,4-Cl_2	112.0	25	EtOH	$C_{21}H_{18}Cl_2N_2O_2$
3d	4-CH_3O	4-$CH_3OC_6H_4$	3,5-Cl_2	141.2	51	EtOH	$C_{21}H_{18}Cl_2N_2O_2$
3e	4-CH_3O	4-$CH_3OC_6H_4$	2,5-Cl_2	96.9	69	EtOH	$C_{21}H_{18}Cl_2N_2O_2$
3f	4-CH_3O	4-$CH_3OC_6H_4$	2,6-Cl_2	110.7	60	EtOH	$C_{21}H_{18}Cl_2N_2O_2$ [l]
2x	2-CF_3	CH_3	2,4-Cl_2	48.4	66	EtOH/H_2O	$C_{15}H_{11}Cl_2F_3N_2$
2y	2-CF_3	CH_3	2,3-Cl_2	99.1	43	EtOH/H_2O	$C_{15}H_{11}Cl_2F_3N_2$ [m]
2z	2-CF_3	CH_3	2-CH_3, 3-Cl	119.4	67	EtOH	$C_{16}H_{14}ClF_3N_2$ [n]
2aa	2-CF_3	CH_3	2-CH_3, 4-Cl	93.3	69	EtOH	$C_{16}H_{14}ClF_3N_2$
2bb	4-C_2H_5O	CH_3	2,3-Cl_2	119.9	74	EtOH	$C_{16}H_{16}Cl_2N_2O$
2cc	4-C_2H_5O	CH_3	3,5-Cl_2	136.0	80	EtOH	$C_{16}H_{16}Cl_2N_2O$
2dd	4-C_2H_5O	CH_3	3,4-Cl_2	111.7	46	EtOH	$C_{16}H_{16}Cl_2N_2O$
2ee	4-CN	CH_3	2,4,6-Cl_3	160.5	81	EtOH/DMF	$C_{15}H_{10}Cl_3N_3$
2ff	4-CN	CH_3	4-Br	178.7	68	EtOH/DMF	$C_{15}H_{12}BrN_3$
2gg	4-NO_2	CH_3	2,4-$(NO_2)_2$	264.8[o]	72	dioxane/DMF	$C_{14}H_{11}N_5O_4$
2hh	4-NO_2	CH_3	H	136.7[p]	87	EtOH/H_2O	$C_{14}H_{13}N_3O_2$
2ii	4-NO_2	CH_3	2-CH_3, 4-Cl	182.1	90	EtOH/H_2O	$C_{15}H_{14}ClN_3O_2$ [q]
2jj	4-NO_2	CH_3	2-CH_3, 3-Cl	187.9	91	EtOH/H_2O	$C_{15}H_{14}ClN_3O_2$
2kk	4-CN	CH_3	2,4-Cl_2	205.4	85	EtOH/DMF	$C_{15}H_{11}Cl_2N_3$
2ll	4-CN	CH_3	2,3-Cl_2	163.8	84	EtOH/DMF	$C_{15}H_{11}Cl_2N_3$
2mm	4-CN	CH_3	3,5-Cl_2	226.0	78	EtOH/DMF	$C_{15}H_{11}Cl_2N_3$
2nn	4-CN	CH_3	2,6-Cl_2	127.6	87	EtOH/DMF	$C_{15}H_{11}Cl_2N_3$
2oo	4-CN	CH_3	2,5-Cl_2	157.4	70	EtOH/DMF	$C_{15}H_{11}Cl_2N_3$
2pp	4-CN	CH_3	3,4-Cl_2	193.3	72	EtOH/DMF	$C_{15}H_{11}Cl_2N_3$
2qq	3-CF_3	CH_3	2,4-Cl_2	111.6	91	EtOH	$C_{15}H_{11}Cl_2F_3N_2$
2rr	4-Cl	CH_3	2,4-Cl_2	136.6[r]	75	EtOH/DMF/H_2O	$C_{14}H_{11}Cl_3N_2$
2ss	4-Cl	CH_3	2,3-Cl_2	131.1	78	EtOH/DMF/H_2O	$C_{14}H_{11}Cl_3N_2$
2tt	4-Cl	CH_3	3,5-Cl_2	126.6	50	EtOH/H_2O	$C_{14}H_{11}Cl_3N_2$
2uu	4-Cl	CH_3	2,5-Cl_2	80.1	70	EtOH/DMF/H_2O	$C_{14}H_{11}Cl_3N_2$
2vv	4-Cl	CH_3	4-CH_3, 3-Cl	97.3	62	EtOH	$C_{15}H_{14}Cl_2N_2$
2ww	4-Cl	CH_3	2-CH_3, 4-Cl	124.9	78	EtOH	$C_{15}H_{14}Cl_2N_2$ [s]
2xx	4-F	CH_3	2,4-Cl_2	114.5	87	EtOH/H_2O	$C_{14}H_{11}Cl_2FN_2$
2yy	4-F	CH_3	2,3-Cl_2	144.6	83	EtOH/H_2O	$C_{14}H_{11}Cl_2FN_2$
2zz	4-F	CH_3	3,4-Cl_2	109.3	76	EtOH/H_2O	$C_{14}H_{11}Cl_2FN_2$
4a	H	CF_3	2,6-Cl_2	100.2	10	Skelly B	$C_{14}H_9Cl_2F_3N_2$
4b	H	CF_3	2,3-Cl_2	93.1	65	Skelly B	$C_{14}H_9Cl_2F_3N_2$
4c	H	CF_3	2,4-Cl_2	45.8	74	MeOH	$C_{14}H_9Cl_2F_9N_2$
4d	H	CF_3	2,5-Cl_2	81.0	66	Skelly B	$C_{14}H_9Cl_2F_3N_2$
4e	H	CF_3	3,4-Cl_2	81.3	75	Skelly B	$C_{14}H_9Cl_2F_3N_2$
4f	H	CF_3	3,5-Cl_2	104.7	76	Skelly B	$C_{14}H_9Cl_2F_3N_2$
4g	H	CF_3	2-CH_3, 3-Cl	108.2	71	Skelly B	$C_{15}H_{12}ClF_3N_2$
4h	H	CF_3	2-CH_3, 4-Cl	74.8	62	Skelly B	$C_{15}H_{12}ClF_3N_2$

[a] Unless otherwise indicated, analyses for the elements C, H, and N were obtained within ±0.4% of the theoretical values. All compounds had reasonable IR spectra. [b] N: calcd, 9.56; found, 9.15. [c] C: calcd, 61.43; found, 60.79. [d] C: calcd, 61.43; found, 60.91. [e] C: calcd, 59.31; found, 58.90. [f] C: calcd, 50.38; found, 49.93. [g] Literature[1] mp 125–128 °C. [h] Literature[9] mp 156–157 °C. [i] N: calcd, 8.76; found, 8.24. [j] C: calcd, 56.64; found, 56.19. [k] C: calcd, 62.84; found, 62.29. [l] C: calcd, 62.84; found, 62.33. [m] C: calcd, 51.87; found, 51.41. [n] C: calcd, 58.81; found, 58.33. [o] Literature[11] mp 268–269 °C. [p] Literature[12] mp 132 °C. [q] N: calcd, 13.84; found, 14.28. [r] Literature[14] mp 150 °C. [s] N: calcd. 9.56; found. 9.14.

Table II. Anthelmintic Evaluation of Benzoyl Chloride Phenylhydrazones (1) in the Mouse



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no.	X	Y	dose, (mg/mouse)/day	% clearance[a] *S. obvelata*	% clearance[a] *N. dubius*	% clearance[a] *H. nana*
1a	4-CH_3	H	0.25	100	20	
1b	H	H	0.45		100	40
1c	4-Cl	H	6.1	toxic		
1d	3,4-Cl_2	H	7.5		100	
1e	4-NO_2	H	7.5		0	0
1g	3-CF_3	H	1.87		0	75
1h	4-I	H	1.87	100	80	
1i	3-CH_3	H	0.12		0	0
1j	3-Cl	H	1.87	100	100	
1k	4-$(CH_3)_2CH$	H	0.47	0	0	
1l	2,4-Cl_2	H	0.47	0	0	
1m	2-Cl, 4-NO_2	H	7.5	0	0	
1n	2-Cl	H	1.87	20	40	
1o	3,5-Cl_2	H	0.57	25	0	
1p	2,3,4,5,6-F_5	H	0.45	0	0	
1q	4-Br	H	0.47		100	100
1u	H	4-NO_2	7.5		0	
1w	H	2,4,6-Cl_3	1.87	20	80	
1x	H	2-CH_3	0.47	0	100	
1y	H	4-Cl	0.47	80	100	
1bb	H	2,4,5-Cl_3	3.75	33	0	
1ee	H	2-CH_3, 4-Cl	7.5	0	0	
1nn	3,4-Cl_2	2,4,6-Cl_3	7.5		0	0
1oo	2,6-Cl_2	2,4,6-Cl_3	7.5		0	0
1vv	4-SCH_3	H	1.87	100	100	
1ss	3-Cl	2,4,6-Cl_3	7.5	100	50	
1uu	3-CH_3, 4-NO_2	H	3.75	100	80	
1tt	4-Br	2,4,6-Cl_3	3.75	40	0	
1cc	H	2,4-Br_2	0.32	0	0	
1dd	4-C_4H_9O	H	1.5	100		100

[a] Five mice treated 4 days orally; no values cited means there were no parasites.

chemical and physical characterization data for **3** are also found in Table I.

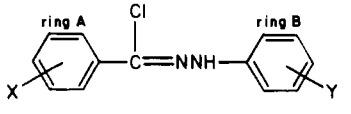
Synthesis of 2,2,2-trifluoroacetophenone hydrazones (**4**) was accomplished by a modification of the method of Simons[5] and Sykes.[6] Except for the 2,4-dinitrophenylhydrazone of 2,2,2-trifluoroacetophenone, all the compounds prepared in this series are novel. The chemical and physical characterization data are found in Table I. The 2,4-dinitrophenylhydrazone of 2,2,2-trifluoroacetophenone has been reported by Simons as having mp 94.5–95.5 °C and by Sykes as having mp 106–107.5 °C. From our studies (see Experimental Section) we believe the compound reported by both Simons and Sykes is the syn isomer (isomer A, mp 106.6 °C) **4k**. It is probable that we were able to isolate both the syn and the anti isomer **4j** and **4k** respectively, because we worked up the reaction product after a 24-h reaction rather than 1 week as did both Simons and Sykes. At 24 h both the kinetically favored product **4k** and the thermodynamically favored product **4j** were present. An interpretation of our observations is represented in Scheme III. Isomer A and isomer B differ significantly in melting points and in their ultraviolet and infrared spectra but give, within experimental error, identical combustion analyses for $C_{14}H_9F_3N_4O_4$. Isomer B is readily converted to isomer A in alcohol containing a trace of mineral acid.

The other hydrazones of 2,2,2-trifluoroacetophenone (**4a–i**) were isolated in only one isomeric form. Apparently in these situations the kinetically controlled product, the anti isomer, is also the thermodynamically favored product.

The bis(phenylhydrazones) of phenylglyoxal (**5a–c**) were prepared by reacting the phenylhydrazine with the α,α-dichloroacetophenone.

Discussion

Structure–Activity Relationships. We, like many other investigators, use a laboratory animal as the host for initial or primary screening of chemicals for anthelmintic effects. Many of our benzoyl chloride phenylhydrazones (**1**) were evaluated for activity against three mouse hel-



1

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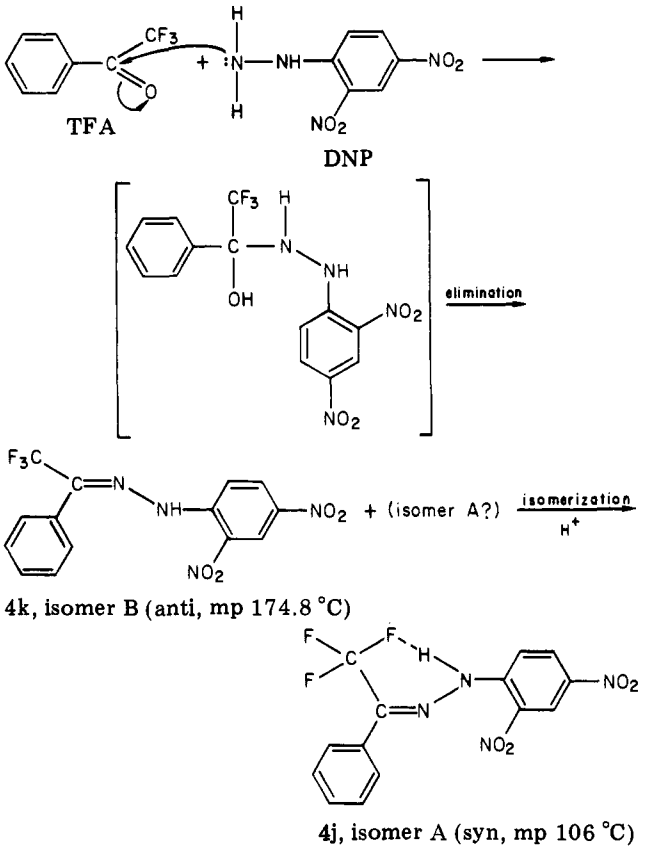
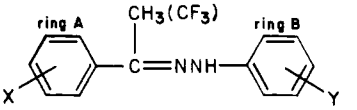
Scheme III



minths, *Syphacia obvelata*, *Nematospiroides dubius*, and *Hymenolepis nana* (Table II). The dosage used was generally the highest dose [up to 7.5 (mg/mouse)/day] which did not elicit toxic effects on the mouse. In some instances, availability of drug was a determining consideration. Examination of the data set (Table II) reveals no tendencies for any one species to be more susceptible or refractory than the other to this class of compounds. The most active compounds in the mouse model are **1d,h,j,q,y,dd,vv,uu**. The structural features required to give the superior anthelmintic activity in the benzoyl chloride phenylhydrazone series are meta and/or para halogen, alkoxy, alkyl, or alkylthio substituent(s) in ring A. These groups have moderately positive π and slightly positive or negative σ. The high activity of the disubstituted compound **1uu** is perplexing. That is, the single substituted analogues of **1uu**, **1e**, and **1i** are two of the least active compounds tested. The inactivity of **1i** could be because of the low dose tested [0.12 (mg/mouse)/day]. The good anthelmintic activity of **1uu** in comparison to **1e** is possibly caused by a moderation of the strong positive σ of the NO_2 moiety by steric inhibition of resonance and slightly negative σ of the methyl group. Noteworthy is the lack of activity of any compound with an ortho substituent in the A ring. Except for the *p*-chloro compound **1y** and *o*-methyl analogue **1x**, any substituent in the B ring gave compounds with little or no activity (**1u,ee**, etc). Multiple substitution in the B ring like in the A ring decreases activity. There does not appear to be the detrimental ortho effect operating in the B-ring substitution as is apparent in the A-ring case.

One of the primordial questions of this study was whether the acid chloride fragment or "active" chlorine is a necessary structural feature for anthelmintic activity in the benzoyl chloride phenylhydrazones. To attempt to answer this question, we replaced the chlorine ($+\sigma^*$, $+\pi$) of **1** with a methyl group ($\sigma^* = 0$, $+\pi$) to afford **2** and then



2 (4)

a trifluoromethyl moiety ($+\sigma^*$, $+\pi$) to give **4**. Other chlorine variations included replacement with a 4-methoxyphenyl (**3**) and a phenylhydrazone methine group (**5**). Anthelmintic evaluation of these compounds revealed a remarkable lack of activity when compared to our candidate benzoyl chloride phenylhydrazone series. Unfortunately, most of the B-ring unsubstituted compounds (compounds best in series **1**) in series **2** and **3** were unstable and, therefore, not tested. Replacement of the chlorine with a *p*-methoxyphenyl (**3**) or phenylhydrazonomethine moiety (**5**) also resulted in inactive compounds.

Having detected a series of compounds (**1**) active against helminths of a laboratory animal, our next series of experiments involved evaluation of the benzoyl chloride phenylhydrazones (**1**) against helminths of the dog. The results of these studies are found in Table III.

In general, the substituents and substituent patterns of the benzoyl chloride phenylhydrazones (**1**), which gave highly active compounds in the mouse model, also gave the best anthelmintic activity in the dog. The most active compounds have a single methyl (**1a**), trifluoromethyl (**1g**), alkoxy (**1dd**), or methylthio (**1vv**) in the para or meta position of ring A. That is, substituents having positive σ with positive π or slightly negative σ with positive π enhance anthelmintic effects. Any substitution of the B ring or disubstitution of A ring gave compounds with inferior anthelmintic activities.

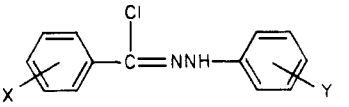
The benzoyl chloride phenylhydrazones (**1**) were evaluated in sheep harboring a broad spectrum of helminths. Review of the sheep anthelmintic data found in Table IV suggests that this class of compounds had a wide spectrum of anthelmintic activities. The compounds most active against helminths of the mouse and dog were also found to be efficacious for treatment of gastrointestinal parasites in sheep (i.e., **1a–c,g,dd,vv**). Compounds where the A ring was unsubstituted or substituted with groups with $+\pi$ and slightly $-\sigma$ or $+\sigma$ had the highest and most broad-spectrum anthelmintic activity. Likewise, the substituents and/or substitution patterns which gave the inferior anthelmintic effects in the dog and mouse were also less efficacious in the sheep.

An important question in using a model in chemotherapeutic (such as anthelmintic) evaluations is whether or not the activities of the model correlate with results or activities in the target animals for which one is designing compounds. In the present study we tested ten compounds against helminths of the mouse, dog, and/or sheep. We found five (**1dd,vv,w,g,a**) active in the mouse and also in the dog and sheep. One compound (**1p**) was inactive against mouse helminths but active against dog and sheep worms. Another compound (**1u**) was inactive against mouse worms and then also inactive against the helminths in dogs and sheep. Three other compounds (**1b,h,uu**) were found active in the mouse screen and then tested either in the dog or sheep. All three of these were also active in the target host. In summary, we found for the benzoyl chloride phenylhydrazone series the mouse model to be generally predictive of anthelmintic results one could expect in dogs and sheep.

Experimental Section

Infrared spectra were measured with a Perkin-Elmer 256 spectrophotometer. H^1 NMR spectra were recorded on a Varian EM-300X. Melting points were obtained with a Mettler FP1

Table III. Anthelmintic Evaluation of Benzoyl Chloride Phenylhydrazones (1) in Dogs



1

no.	X	Y	oral dose/ treatment	% reduction in EPG[a]			
				Ancylostoma	*Uncinaria*	*Toxascaris*	*Trichuris*
1a	4-CH_3	H	200/1	100	100	100	100
1a	4-CH_3	H	250/1	100	100		
1a	4-CH_3	H	275/1	100	99.8		
1g	3-CF_3	H	100/1	100	100	100	
1h	4-I	H	100/1		41	0	
1h	4-I	H	200/1		63	100	
1p	2,3,4,5,6-F_5	H	75/1		100	71.4	
1u	H	4-NO_2	100/1	0	0		
1w	H	2,4,6-Cl_3	175/1	31	26		
1w	H	2,4,6-Cl_3	200/1	87	59	67	
1ii	H	3,5-Cl_2	50/1		57	40	
1jj	H	3,4-Cl_2	100/1	0	0	0	
1pp	2-CH_3	2,4,6-Cl_3	100/1	0	20	100	
1dd	4-C_4H_9O	H	10/2	92	75		
1dd	4-C_4H_9O	H	20/1	86	86		
1dd	4-C_4H_9O	H	20/3	0	100		
1dd	4-C_4H_9O	H	25/1	100	67		
1uu	3-CH_3, 4-NO_2	H	100/1		0		
1uu	3-CH_3, 4-NO_2	H	200/1		55		
1vv	4-SCH_3	H	150/1	84	91	100	
1vv	4-SCH_3	H	50/1	91	86	69	
1vv	4-SCH_3	H	100/1	94	99		

[a] No values cited means there were no paracites.

melting point apparatus (heating rate of 1 °C/min), and melting ranges were measured with a Thomas-Hoover melting point apparatus. Elemental analyses were performed by the Physical and Analytical Chemistry Department of The Upjohn Company.

2,2,2-Trifluoroacetophenone 1-Naphthylhydrazone (4i). To 105 mL of 50% H_2SO_4 was added 5.84 g (0.03 mol) of 1-naphthylhydrazine hydrochloride, followed by 105 mL of EtOH with stirring. To this solution was added 5.22 g (0.03 mol) of 2,2,2-trifluoroacetophenone, and the mixture was stirred at ambient temperature for 48 h. The solid which separated was collected, washed with H_2O, and dried to afford 7.0 g of rust-colored powder. The crude material was crystallized from Skellysolve B to give 5.28 g (56% of theory[16]) of **4i** as rose-colored needles: mp 109.1 °C; IR ν_{max} 3325 (NH), 3055 and 3015 (aromatic CH), 1600 (C=N), 1582, 1526, and 1486 (aromatic C=C), 1360 (CF_3). Anal. ($C_{18}H_{13}F_3N_2$) H, N; C: calcd, 58.78; found, 58.31.

2,2,2-Trifluoroacetophenone 2,4-Dinitrophenylhydrazones (4j and 4k). By the procedure employed to prepare 2,2,2-trifluoroacetophenone 1-naphthylhydrazone, 5.22 g (0.03 mol) of 2,2,2-trifluoroacetophenone and 5.94 g (0.03 mol) of 2,4-dinitrophenylhydrazine furnished 10.0 g (94% of theory) of crude 2,4-dinitrophenylhydrazone. The crude material was suspended in 100 mL of boiling absolute EtOH. The mixture was filtered. The insoluble material (isomer B) was washed with 2 × 25 mL of absolute EtOH. The combined filtrate and washings were chilled. The solid which deposited was collected and dried: yield 4.18 g (39% of theory) of isomer A (**4j**) as yellow crystals; mp 106.6 °C; IR ν_{max} 3290 (NH), 3110, 3070 (aromatic CH), 1621 (C=N), 1600, 1571, 1547, 1531, 1514, 1507, and 1494 (aromatic C=C), 1344 (NO_2). Anal. ($C_{14}H_9F_3N_4O_4$) C, H; N: calcd, 15.82; found, 16.29.

The EtOH-insoluble material from above was crystallized from EtOAc to afford 2.3 g (22% of theory) of isomer B (**4k**) as yellow platelets: mp 174.8 °C; IR ν_{max} 3310 (NH), 3120 and 3110 (aromatic CH), 1621 (C=N), 1600, 1590, 1571, 1552, 1512, 1505, and 1495 (aromatic C=C), 1345 (NO_2). Anal. ($C_{14}H_9F_3N_4O_4$) C, H, N.

4'-Methylacetophenone 3,4-Dichlorophenylhydrazone (2a). A mixture of 4.03 g (0.03 mol) of 4'-methylacetophenone, 6.41 g (0.03 mol) of 3,4-dichlorophenylhydrazine hydrochloride, and 100 mL of EtOH was refluxed for 1 h. The solid which separated was collected and then recrystallized from EtOH to afford 6.31 g (72% of theory) of **2a** as white cotton-like needles: mp 123.4 °C; IR ν_{max} 3375 (NH), 3020 (aromatic CH), 2918 (methyl CH), 1599 (C=N), 1495 and 1455 (aromatic C=C), 1270 and 1240 (CN). Anal. ($C_{15}H_{14}Cl_2N_2$) C, H; N: calcd, 9.56; found, 9.15. Synthesis of **2a** typifies the method used to prepared series **2** found in Table I.

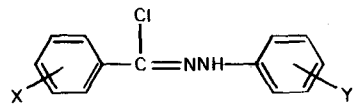
4,4'-Dimethoxybenzophenone 2,4-Dichlorophenylhydrazone (3a). A mixture of 7.26 g (0.03 mol) of 4,4'-dimethoxybenzopheneone, 6.41 g (0.03 mol) of 2,4-dichlorophenylhydrazine hydrochloride, 50 mL of absolute EtOH, and 50 mL of MeOH was stirred at room temperature for 18 h. The mixture was heated to boiling and diluted with sufficient DMF to give a solution. The hot solution was slowly cooled to give, after collecting and drying, 8.39 g (70% of theory) of **3a** as large amber crystals: mp 161.7 °C; IR ν_{max} 3345 (NH), 3065, 3035, and 3010 (aromatic CH), 2965, 2935, and 2835 (methyl CH), 1608 (C=N), 1596, 1574, 1522, 1496, and 1464 (aromatic C=C), 1291, 1271, and 1243 (CN and aromatic CO), 1029 (aliphatic CO). Anal. ($C_{21}H_{18}Cl_2N_2O_2$) H, N; C: calcd, 62.84; found, 62.29. Preparation of **3a** in representative of the procedure used to synthesize series **3** found in Table I.

2,4-Dichlorophenylglyoxal Bis(2,6-dichlorophenylhydrazone) (5a). To 50 mL of 85% H_3PO_4 was added 6.4 g (0.03 mol) of 2,6-dichlorophenylhydrazine hydrochloride at 75 °C. Absolute EtOH (50 mL) was then added with cooling. To this solution was added 7.74 g (0.03 mol) of 2,2,2',4'-tetrachloroacetophenone at room temperature, and the mixture was stirred for 5 days. The reaction mixture was diluted to 500 mL volume with H_2O. The solid which separated was collected and recrystallized from EtOH–Skellysolve B: yield 3.57 g (46% of theory) of **5a** as yellow crystals; mp 103.9 °C; IR ν_{max} 3320 and 3310 (NH), 3090 (aromatic CH), 1588 (C=N), 1577, 1535, 1496, 1474, and 1455 (aromatic C=C), 1278 and 1250 (CN). Anal. ($C_{20}H_{12}Cl_6N_4$) C, H; Cl: calcd, 40.98; found, 41.78; N: calcd, 10.75; found, 9.99.

2,4-Dichlorophenylglyoxal Bis(3,5-dichlorophenylhydrazone) (5b). By the procedure used to prepare **5a**, 4.27 g (0.02 mol) of 3,5-dichlorophenylhydrazine hydrochloride and 5.46 g (0.02 mol) of 2,2,2',4'-tetrachloroacetophenone gave, after recrystallizing from EtOAc–Skellysolve B, 1.80 g (35% of theory)

(16) No attempt was made to optimize yields. The yields reported are isolated yield.

Table IV. Anthelmintic Evaluation of Benzoyl Chloride Phenylhydrazones (1) in Sheep



1

no.	X	Y	dose, mg/kg	rte	% reduction of EPG[a]								
					Haemonchus	*Ostertagia*	*Trichostrongylus*	*Oesophagostomun*	*Trichuris*	*Strongyloides*	*Nematodirus*	*Moniezia*	*Cooperia*
1a	4-CH_3	H	100	po	84	96	73	100	29	99	100		100
1a	4-CH_3	H	150	po	100	100	98	79	25	100	100	100	
1a	4-CH_3	H	200	po	100	100	100	100	0	37	100		
1a	4-CH_3	H	300	po	100	100	100	100	0	100	100		
1a	4-CH_3	H	400	po	100	100	100		0	100	100		
1b	H	H	100	po	87	95	100	89	0	100	100		
1c	4-Cl	H	100	po	100	100	99	100	0	100	100		
1g	3-CF_3	H	100	po	98	98	97	100	35	98	100		100
1g	3-CF_3	H	200	po	99	100	99	100	0	100	100	100	100
1g	3-CF_3	H	250	po	84	86	71	91	57	59	29		
1g	3-CF_3	H	350	po		100	100	100	0	100	100		
1g	3-CF_3	H	400	po	100	100	100	100	62	100	100	100	100
1g	3-CF_3	H	450	po	100	100	100		0	100			
1j	3-Cl	H	150	po	toxic								
1p	2,3,4,5,6-F_5	H	200	po	85	93	0	100	10	93			
1p	2,3,4,5,6-F_5	H	400	po	96	95	96		88	99			
1u	H	4-NO_2	100	po	0	33	57	0	100	58	0	100	
1w	H	2,4,6-Cl_3	250	po	98	100	98	95	63	95	82		
1w	H	2,4,6-Cl_3	325	po	99	98	33		0	79			
1w	H	2,4,6-Cl_3	400	po	85	100	77		14			100	100
1w	H	2,4,6-Cl_3	450	po	94	100	100	0	29	100	63		
1dd	4-C_4H_9O	H	100	po	100	100	100	100	100	32			
1dd	4-C_4H_9O	H	200	po	100	100	100	100		100			
1dd	4-C_4H_9O	H	150	ip	81		0	71		0			
1ff	H	2,3,4-Cl_3	100	po	0	0	0	0	0	95			89
1ii	H	3,5-Cl_2	100	po	97	100	100	0	100	45			
1jj	H	3,4-Cl_2	100	po	toxic								
1pp	2-CH_3	2,4,6-Cl_3	100	po	85	67	14	11	0	85			33
1vv	4-SCH_3	H	100	po	98	98	98	100	25	100	100		
1vv	4-SCH_3	H	300	po	100	100	100		73	100	100		
1vv	4-SCH_3	H	400	po	100	100	100	100	38	100	100	100	100

[a] No values cited means no parasites.

of **5b** as green fine crystals; mp 227.6 °C dec; IR ν_{max} 3300 (NH), 3085 (aromatic CH), 1599 (C=N), 1568, 1553, 1541, 1518, 1488, and 1450 (aromatic C=C), 1270 and 1233 (CN). Anal. ($C_{20}H_{12}Cl_6N_4$) C, H, N.

2,4-Dichlorophenylglyoxal Bis(2,5-Dichlorophenylhydrazone) (5c). By the method used to synthesize **5a**, 4.27 g (0.22 mol) of 2,5-dichlorophenylhydrazine hydrochloride and 5.16 g (0.02 mol) of 2,2,2',4'-tetrachloroacetophenone gave, after recrystallizing from benzene–Skellysolve B, 2.24 g (27% of theory) of **5c** as orange fine crystals: mp 208.2 °C; IR ν_{max} 3350 and 3355 (NH), 3100 (aromatic CH), 1590 (C=N), 1547, 1523, 1500, 1474, and 1457 (C=C stretch), 1264, 1255, and 1241 (CN). Anal. ($C_{20}H_{12}Cl_6N_4$) C, H, N.

***m*-Chlorobenzoyl Chloride 2,4,6-Trichlorophenylhydrazone** (1ss). To a suspension of 11.52 g (0.05 mol) of *m*-chlorobenzaldehyde phenylhydrazone in 300 mL of glacial HOAc at ambient temperature was added 11.7 mL (0.25 mol) of Cl_2 with stirring. An exothermic reaction ensued. The mixture was cooled to 20 °C, stirred for 0.5 h, and then filtered. The crude product was crystallized from Skellysolve B to afford 13.4 g (73% of theory) of **1ss** as white crystals, mp 127–128 °C. Anal. ($C_{13}H_7Cl_5N_2$) C, H, Cl, N.

***p*-Toluoyl Chloride Phenylhydrazone** (1a). To 5.89 g (0.026 mol) of *p*-toluic acid phenylhydrazide in 100 mL of CCl_4 was added 6.25 g (0.03 mol) phosphorus pentachloride. The mixture was cautiously heated to reflux. After 2 h, the mixture was cooled and then chilled in an ice bath. To this mixture was added dropwise 13.18 g (0.14 mol) of phenol in 60 mL of CCl_4 with stirring over 0.5 h. Stirring was continued at 0 °C for 0.75 h, and then the mixture was concentrated in vacuo to dryness. The residue was suspended in cold (0 °C) MeOH and filtered. The crude product was dissolved in 25 mL of hot CH_2Cl_2, diluted with 25 mL of Skellysolve B, and concentrated to 30 mL total volume. Cooling gave yellow plates: yield 4.36 g (68% of theory); mp 134–136 °C. Anal. ($C_{14}H_{13}ClN_2$) C, H, Cl, N.

Anthelmintic Evaluations. The various compounds were evaluated for preliminary anthelmintic activity in mice and, if active, also in dogs and sheep.

Mice naturally parasitized with *Syphacia obvelata* were experimentally infected with *Nematospiroides dubius* and *Hymenolepis nana*. After a prepatency period of 2 weeks, the mice were allotted to treated and control groups. The compounds were formulated for intraperitoneal and oral treatments by grinding the material with a mortar and pestle and then suspending the finely ground material in a sterile aqueous vehicle consisting of 0.10% carboxymethylcellulose, 0.04% polysorbate 80, and 0.0042% polyparaben. The treatment(s) was then administered to the animals on a milligram per kilogram of body weight basis. Each suspension 0.1 mL, was orally administered via cannula or intraperitoneally administered via a 20-gauge hypodermic needle; each mouse received the treatment once a day for 4 days. Two days after the final treatment, all of the mice were necropsied and examined for the presence or absence of the parasites. Ratios were developed for the treated and control mice based on the absence of the three parasites. Activity was then determined by comparing the ratios of the treated mice and the nontreated controls. The percent clearance was found by subtracting the percent clearance, if any, of the controls from the apparent percent clearance of the treatment groups. A compound found to give 50% or greater clearance of one or more of the mouse parasites was further evaluated for activity in dogs and/or lambs.

Compounds were evaluated in naturally parasitized lambs. Anthelmintic activity was determined by the clinical test method (i.e., significant decrease in posttreatment helminth egg counts). The McMaster technique was used to determine the number of eggs per gram (EPG) of fecal material from each animal. Three pretreatment and three posttreatment egg count determinations were made on each animal. One animal was used for each compound and/or for each dosage regimen for each compound.

Each compound was finely ground with a mortar and pestle and then suspended by sonication in approximately 50 mL of the sterile vehicle as described previously. The suspension was administered orally using a stomach tube. Alternatively, the finely ground test compound was weighed and placed in gelatin capsules. The capsules were administered orally via a balling gun. Compounds active against one or more of the parasites of mice were also evaluated against hookworms and ascarids of dogs. Dogs naturally infected with *Toxascaris leonina* (ascarids) were experimentally infected with a mixed culture of *Ancylostoma caninum* and *Uncinaria stenocephala* larva. Test compounds were finely ground, weighed, and placed in gelatin capsules for oral administration. Three pre- and three posttreatment egg count determinations were made during the course of each experiment. A significant decrease in the posttreatment egg counts was the criteria used to determine activity.

Acknowledgment. We thank N. H. Knight and associates of The Upjohn Co. for performing the elemental analyses.

Supplementary Material Available: Mouse anthelmintic data for phenylhydrazone series **2**, **3**, and **4** are listed (3 pages). Ordering information is given on any current masthead page.

Quantitative Structure–Selectivity Relationships. Comparison of the Inhibition of *Escherichia coli* and Bovine Liver Dihydrofolate Reductase by 5-(Substituted-benzyl)-2,4-diaminopyrimidines

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In our previous publication (Blaney, J. M.; Dietrich, S. W.; Reynolds, M. A.; Hansch, C. *J. Med. Chem.* **1979**, *22*, 614), correlation equations were presented for the inhibition of bovine liver and *Escherichia coli* dihydrofolate reductase (DHFR) by 5-(substituted benzyl)-2,4-diaminopyrimidines. These equations brought out differences in the way these two enzymes interact with substituents, which explain the high selectivity of drugs like trimethoprim. We have tested and further developed these equations in this report. It is of particular interest that our previously published correlation equation for *E. coli* DHFR accurately predicted the potency of a commercial competitor of trimethoprim (tetroxoprim) now in clinical use. We believe that new and effective competitors for trimethoprim can be designed by means of the two correlation equations.

In our recent study[2,3] of the inhibition of DHFR from bovine liver and *E. coli* by benzylpyrimidines I, we formulated the quantitative structure–activity relationships (QSAR) of eq 1 and 2. *C* in these equations is the molar

0022-2623/81/1824-0538$01.25/0