on the metabolism of the compounds and their potential long-term therapeutical efficacy. The present data clearly suggest that factors other than affinity for the estrogen receptor play a major role in determining the potency of antiestrogen compounds. The factors pertain to absorption, transport,[32] and especially metabolism at various sites, including the site of intracellular action. The availability of these new halo-steroidal antiestrogens should be useful for a better understanding of estrogen action at the molecular and cellular level and for the development of an improved therapy of breast and endometrial cancer.

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(33) Kramer, C. Y. *Biometrics* **1956**, *12*, 307.

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Synthesis and Anthelmintic Activity of 3′-Benzoylurea Derivatives of 6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole†

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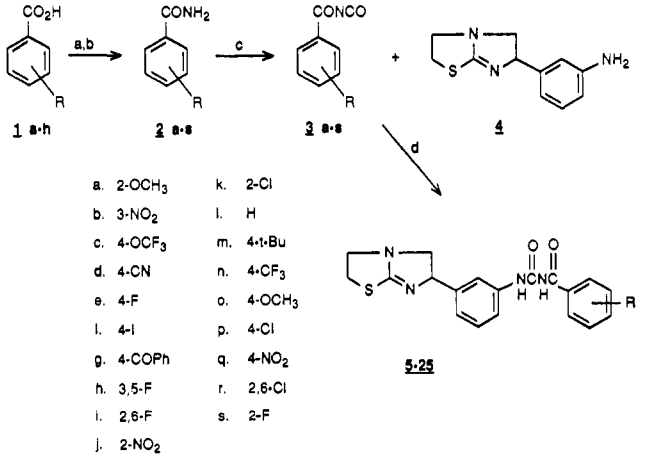
Reaction of 3-amino derivatives of the nematocides tetramisole and levamisole with variously substituted benzoylisocyanates gave a series of benzoylureas I which were tested for activity against helminths and ectoparasites. Compounds bearing 2,6-difluoro and 4-trifluoromethyl substituents had potent nematocidal activity in both mice and sheep. No antiectoparasitic activity was observed.

Parasitic infestations in food-producing animals are economically important in modern agriculture. The development of resistance to several important classes of veterinary anthelmintics[1,2] requires the production of more effective agents with a broad spectrum of activity. Traditionally, endo- and ectoparasitic infections have been treated as separate disease states requiring the administration of different pharmacologic agents. More recently there has been an increased interest in discovering therapeutic anthelmintics which possess both endoparasitic and ectoparasitic activity. The most notable compound to belong to this class is ivermectin which was introduced to the marketplace in 1981. This compound is extremely potent against both nematode and arthropod parasites.[3] These "endectosides" have the potential to be more efficient and economical in the field.

We have attempted to modify the well-known nematocide tetramisole (II)[4] in an effort to obtain a broad-spectrum anthelmintic with antiectoparasitic activity. We were encouraged by the report that certain isothiourea analogues of tetramisole (III) have an increased spectrum of activity and are effective against cestodes and trematodes as well as nematodes.[5] It is also well known that diacylureas such as diflubenzuron (IV), a benzoylurea which inhibits chitin synthesis, are effective for the control of arthropods.[6] We now report on the synthesis and anthelmintic activity of

Scheme I[a]



[a] (a) Oxalyl chloride, CH_2Cl_2, DMF (cat.), reflux; (b) NH_4OH, EtOAc; (c) oxalyl chloride, dichlorethane, reflux; (d) CH_3CN, 70 °C.

† Contribution #815 from the Institute of Organic Chemistry.
‡ Institute of Organic Chemistry.
§ Institute of Cancer and Developmental Biology.
∥ Australian Research Unit.

(1) (a) Prichard, R. K.; Kelly, J. D.; Thompson, H. G. *Vet. Parasitol.* **1978**, *4*, 243. (b) Prichard, R. K.; Hall, C. A.; Kelly, J. D.; Martin, I. C. A.; Donald, A. D. *Aust. Vet. J.* **1980**, *56*, 239.
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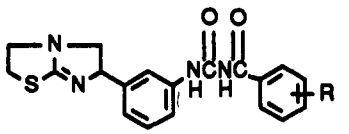
0022-2623/91/1834-1630$02.50/0

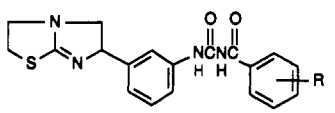
Table I. Anthelmintic Activity of 3′-Benzoylureas in the Mouse



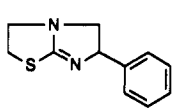
no.	R	*RS* or *S*	% reduction of *N. dubius*[a] 1000[b]	500	250	125	62	31	mol formula[c]	mp, °C	$[\alpha]_D$[d]	% yield[e]
5	2-OCH_3	*S*			0				$C_{20}H_{20}N_4O_3S$	178–179	−52.2	66
6	3-NO_2	*S*			0				$C_{19}H_{17}N_5O_4S \cdot {}^1/_2H_2O$	135 dec	−71.8	26
7	4-OCF_3	*S*			0				$C_{20}H_{17}F_3N_4O_3S$	163–165	−60.4	92
8	4-CN	*S*			0				$C_{20}H_{17}N_5O_2S$	203 dec	−67.3	90
9	4-F	*S*		43	0				$C_{19}H_{17}FN_4O_2S$	203–204	−71.0	63
10	4-I	*S*			0				$C_{19}H_{17}IN_4O_2S$	191–192	−46.9	89
11	4-COPh	*S*	100		0				$C_{26}H_{22}N_4O_3S$	199–200	−62.7	58
12	3,5-F	*R,S*		0					$C_{19}H_{16}F_2N_4O_2S \cdot {}^1/_4H_2O$	186–188		82
13	2,6-F	*S*		100	100	26	0		$C_{19}H_{16}F_2N_4O_2S$	168–169	−64.5	92
14	2,6-F	*R,S*		100	100	76	0		$C_{19}H_{16}F_2N_4O_2S \cdot {}^1/_2H_2O$	173–175		75
15	2-NO_2	*R,S*		0					$C_{19}H_{17}N_5O_4S \cdot {}^1/_2H_2O$	172–174		55
16	2-Cl	*S*			100	56	0		$C_{19}H_{17}ClN_4O_2S \cdot {}^1/_4H_2O$	204–205	−53.9	80
17	H	*R,S*	59	0					$C_{19}H_{18}N_4O_2S \cdot {}^1/_4H_2O$	193–194		85
18	4-t-Bu	*S*			0				$C_{23}H_{28}N_4O_2S \cdot {}^1/_2H_2O$	212–214	−60.3	50
19	4-CF_3	*S*			43	0			$C_{20}H_{17}F_3N_4O_2S$	190–191	−70.5	94
20	4-CF_3	*R,S*		100	100	96	43	0	$C_{20}H_{17}F_3N_4O_2S \cdot HCl \cdot 2H_2O$	170 dec		65
21	4-OCH_3	*R,S*	0						$C_{20}H_{20}N_4O_3S \cdot {}^1/_2H_2O$	196–198		56
22	4-Cl	*R,S*		0					$C_{19}H_{17}ClN_4O_2S \cdot HCl$	225 dec		55
23	4-NO_2	*R,S*	0						$C_{19}H_{17}N_5O_4S \cdot {}^1/_2H_2O$	195–197		85
24	2,6-Cl	*R,S*	56	0					$C_{19}H_{16}Cl_2N_4O_2S \cdot {}^1/_2H_2O$	208–211		65
25	2-F	*S*				95	40	0	$C_{19}H_{17}FN_4O_2S \cdot {}^1/_2H_2O$	155–157	−44.7	68
tetramisole		*R,S*		100		100	0					
levamisole		*S*		100			100	60				
3-NH_2-tetramisole		*R,S*				100	100	63				
3-NH_2-levamisole		*S*				100	100	100				
oxfendazole						100	100	100				

[a] Relative to untreated control. [b] Test compound concentration, parts per million in feed. [c] All compounds analyzed within ±0.4% of calculated values. [d] In DMSO at ca. 0.005 μg/mL, in degrees. [e] From acyl isocyanate.

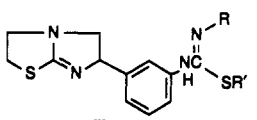
a series of 3′-benzoylurea-substituted tetramisole and levamisole analogues.



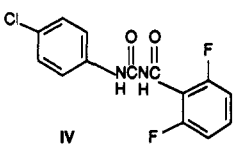
I



II



III



IV

Chemistry

3′-Aminotetramisole **4** was prepared with some modifications of the published procedure of Spicer and Hand.[7,8] The *S* isomer was obtained by hydrolysis of the commercially available isobutyramide butamisole.[9] Acyl isocyanates **3a–s** were prepared as shown in Scheme I. Treatment of substituted benzoic acids **1a–h** with oxalyl chloride in methylene chloride at reflux provided the acid chlorides which upon treatment with ammonium hydroxide in ethyl acetate gave benzamides **2a–h**. These, together with the commercially available benzamides **2i–s**, were converted to the acyl isocyanates **3a–s** according to literature procedures.[10] Condensation of 3′-aminotetramisole or 3′-aminolevamisole with acyl isocyanates **3a–s** in acetonitrile at 70 °C then provided the benzoylureas **5–25** as shown in Scheme I.

Biological Results and Discussion

Compounds were initially tested in an in vivo model in which the compound, mixed in the appropriate concentration in the food, was administered to mice infected with the nematode *Nematospiroides dubius* and the cestode *Hymenolepis nana*. After 18 days of treatment the animals were sacrificed and the intestinal parasite burden was determined (see the Experimental Section). This model is effective in detecting the anthelmintic activity of established agents.[11] Several of the benzoylurea compounds showed potent anthelmintic activity, markedly dependent on the nature of the substituent(s) on the aromatic ring.

The initial lead compound in this series was racemic (2,6-difluorobenzoyl)urea analogue **14**. As shown in Table I this compound was orally effective in reducing the worm burden at a dose down to 125 ppm. Unsubstituted compound **17** and 2,6-dichloro compound **24** showed only modest activity. Monofluoro compound **9** retained some of the activity of the difluoro compound whereas monochloro compound **16** demonstrated increased potency when

(3) Benz, G. W.; Roncalli, R. A.; Gross, S. J. In *Ivermectin and Abamectin*; Campbell, W. C., Ed.; Springer-Verlag: New York, 1989; p 215.
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(6) Van Eck, W. H.; Oppenoorth, F. J. *Integr. Control Insect Pests Neth.* **1980**, 223.
(7) Spicer, L. D.; Hand, J. J. U.S. Patent 3989835, 1976.
(8) Bromination α to the ketone of the protected aminoacetophenone was carried out with pyrrolidone hydrotribromide in THF at reflux. The ring closure, done in sulfuric acid at 0 °C, worked best when the unprotected imine of the thiazoline ring was used.
(9) Butamisole is the active ingredient in the canine anthelmintic Styquin (Haver).
(10) Speziale, A. J.; Smith, L. R. *J. Org. Chem.* **1963**, *28*, 1805.
(11) Brody, G.; Elward, T. E. *J. Parasitol.* **1971**, *57*, 1068–1077.

compared with the dichloro analogue. Other substitution at the 2-position as in compounds **5** and **15** was not beneficial. Several compounds were synthesized with variations at the 4-position (compounds **7–11**, **18–23**, Table I). Racemic 4-(trifluoromethyl)benzoylurea **20** proved to be the most potent compound in the series with 96% reduction of *N. dubius* in the mouse at a dose down to 125 ppm given orally in the feed. Of the 4-halogen-substituted compounds only fluoro analogue **9** showed any activity. 4-Benzoyl analogue **11** also showed reasonable activity. All other substitutions at the 4-position, including trifluoromethoxy (compound **7**), resulted in complete loss of activity. Compounds with substitution at the 3- or 3,5-position(s) (**6** and **12**, respectively) were inactive at the highest doses tested.

We considered the possibility of an in vivo hydrolysis of the acylurea to the parent aminotetramisole as an explanation for activity. However, it seemed unreasonable to expect the observed narrow structure–activity relationship if these compounds were simply serving as aminotetramisole prodrugs. Since the substituents on the benzoyl ring of compounds **7** and **20** have similar σ and π coefficients,[12] little difference in the relative rates of chemical hydrolysis would be expected for these compounds. Biological evaluation, however, demonstrated that compound **20** was one of the most active compounds tested, while no activity was observed for compound **7**.

A pharmacokinetic study using sheep was conducted to determine whether the possible metabolite 3-aminolevamisole could be observed in the blood plasma following an intraruminal injection of (2,6-difluorobenzoyl)urea **13**. Sheep were separated into three groups of seven with each group receiving varying doses of compound **13** (10.9 or 16.4 mg/kg of the hydrochloride salt or 15 mg/kg of the free base). HPLC analysis of the blood plasma was then performed at different time intervals. The 15 mg/kg dose gave the highest peak plasma level of **13** (5 μg/mL), which was observed between 9 and 12 h with complete elimination at 48–72 h post treatment. From the total of 21 animals only a small amount of 3-aminolevamisole was detected in one animal.[13] The observation of circulating blood plasma levels of the benzoylurea without the presence of any hydrolyzed material (3-aminolevamisole) is strong evidence that these compounds are intrinsically active.

It is known that tetramisole is a cholinergic agonist and that this agonism is responsible for its anthelmintic effect.[14] Furthermore, it is known that the 6-*S* isomer, levamisole, is responsible for the activity of the racemate.[15] As noted in Table I, several of the analogues were prepared as the *S* isomers and would, a priori, be expected to show greater potency than their racemic counterparts.[16] Although the in vivo mouse screen was sensitive enough to show a near doubling of potency between levamisole and tetramisole, we were unable to demonstrate a similar increase in activity of the chiral (2,6-difluorobenzoyl)urea derivative **13** over the racemic compound **14**. In fact the *S* isomer gave a smaller reduction in worm burden than did the racemic material when the two were tested in the same assay. This unexpected result may be due to a difference in the bioavailability of the racemic and enantiomerically pure forms of this compound. On the other hand, these results might indicate that the benzoylurea portion of (*R,S*)-**14** has intrinsic anthelmintic activity. This postulate is supported by the above described finding that these compounds are not simply serving as prodrugs of 3-aminolevamisole. However, the benzoylurea diflubenzuron, which resembles the non-levamisole portion of **14**, is inactive in the mouse assay at 1000 ppm.[17]

On the basis of the promising results obtained for some of the compounds in the mouse assay, several of the compounds were examined for activity in a sheep model. Sheep were infected with an intraruminal inoculum of third stage larvae (L_3) of *Haemonchus contortus*, *Ostertagia circumcincta*, and *Trichostrongylus colubriformis*. After 28 days the test compound was administered via intraruminal injection, and at day 35 the parasite burdens were determined (see the Experimental Section). Racemic 2,6-difluoro analogue **14** was effective against the three nematode species after a single oral dose of 30 mg/kg, but activity decreased below commercially significant levels at 15 mg/kg. As would be predicted, the compound was effective against benzimidazole-resistant strains of *H. contortus* and *T. colubriformis* but considerably less active against a levamisole-resistant strain of *O. circumcincta*. As in the mouse screen, it was not possible to demonstrate in sheep any increase in anthelmintic activity for *S* isomer **13** relative to its racemic counterpart **14**. When **13** was tested at 15 mg/kg, its efficacy was very similar to that of the racemate at the same dose.

Chiral compounds (*S*)-**13** and (*S*)-**19** were assayed for contact toxicity at 100 ppm against the sheep louse *Damalia ovis*[18,19] and at 1000 and 100 ppm against larvae and adults of the sheep blowfly *Lucilia cuprina*.[20] No insecticidal activity was observed. Since insecticides of the diflubenzuron type might be expected to affect chitin-dependent processes such as egg production and hatching, the compounds were tested for ovicidal properties. However, after a methanolic solution of the test compound (1 μM) was applied to the abdomen of gravid females of *L. cuprina*[20,21] with a Burkard microapplicator, the subsequent egg hatch was found to be normal.

Thus, although the benzoylurea derivatives of levamisole had broad-spectrum nematocidal activity, the relatively low potency and lack of insecticidal activity did not encourage any further studies in this series.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Microanalyses were performed on a Control Equipment Corp. Model 240XA by the Syntex Analytical Department. ^1H and ^{13}C NMR spectra were measured on a Bruker WM 300 and AM 500 spectrometer in $CDCl_3$ or Me_2SO-d_6 solution referenced to internal tetra-

(12) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
(13) A detailed account of this study is currently being prepared for publication.
(14) Lewis, J. A.; Wu, C.-H.; Levine, J. H.; Berg, H. *Neuroscience* **1980**, *5*, 967.
(15) Bullock, M. W.; Hand, J. J.; Waletzky, E. *J. Med. Chem.* **1968**, *11*, 169.
(16) The optical purities of the (*S*)-acylureas could not be measured directly. However, since the specific rotation of the (*S*)-3-aminolevamisole agreed with the published value (ref 22) and racemization during acylurea formation is very unlikely, we believe the compounds to be stereochemically homogenous. The observed specific rotations (Table I) support this conclusion.
(17) Unpublished data from these laboratories.
(18) The bioassays were performed at the Entomology Division, Biological and Chemical Research Institute, Department of Agriculture and Fisheries, New South Wales, Australia.
(19) Levot, G. W.; Hughes, P. V. *J. Aust. Entomol. Soc.*, in press.
(20) Levot, G. W.; Schipp, E. *Ned. Entomol. Ver. Amsterdam* **1983**, *34*, 58.
(21) Hughes, P. V.; Levot, G. W. *Vet. Parasitol.* **1987**, *24*, 275.
(22) Spicer, L. D.; Hand, J. J., U.S. Patent 3673205, 1972.

methylsilane. Infrared spectra were measured on a Nicolet 5 PC FT-IR spectrometer. Silica gel chromatography was performed with 70-230 mesh (Merck) silica gel. Acetonitrile and dichloroethane were dried over 5-Å molecular sieves and all reactions were carried out under a dry nitrogen atmosphere.

Chemistry. General Procedure for Preparation of Acyl Isocyanates 3a–s; 3,5-Difluorobenzamide (2h). To a solution of 3,5-difluorobenzoic acid (5.0 g, 31.6 mmol) in dry dichloromethane (50 mL) at 0 °C under an N_2 atmosphere was added dropwise oxalyl chloride (4.4 mL, 50.6 mmol) followed by 2–3 drops of dimethylformamide. The ice bath was removed, the reaction heated to reflux for 4–5 h and cooled, and the solvent removed in vacuo to afford a light brown oil. This was placed under reduced pressure to remove residual oxalyl chloride. The acid chloride was taken up in dry ethyl acetate (50 mL) and added dropwise to an ice-cold solution of ethyl acetate (250 mL) containing concentrated ammonium hydroxide (50 mL). After addition, the reaction was stirred cold for 10 min, and the layers were separated. The ethyl acetate layer was washed twice with H_2O (100 mL) and twice with brine (75 mL), dried over $MgSO_4$, and evaporated to afford the crude product. This was triturated with hexane to afford 4.4 g (89%) of a white solid (single spot by TLC 95:5:0.1 $CH_2Cl_2/CH_3OH/NH_4OH$). The crude benzamides were converted directly to the acyl isocyanates. All other benzamides were prepared in a similar fashion and used directly without further purification.

3,5-Difluorobenzoyl Isocyanate (3h). To a solution of **2h** (4.4 g, 28 mmol) in dichloroethane (100 mL) was added oxalyl chloride (4.2 mL, 47.6 mmol) dropwise. The mixture was heated to reflux for 16–20 h and the solvent removed by distillation (bp 83 °C at 760 mmHg). The residual acyl isocyanates, in general, were stable enough to be purified by reduced pressure distillation, but this was found to not be necessary. All of the acyl isocyanates were prepared by this method.

General Procedure for Preparation of Acylureas 5–25: 1-(3,5-Difluorobenzoyl)-3-[3-[6-(2,3,5,6-tetrahydroimidazo-[2,1-*b*]thiazolyl)]phenyl]urea (12). To a solution of 3-aminolevamisole or 3-aminotetramisole (1.0 g, 4.56 mmol) in dry acetonitrile (50 mL) was added **3h** (1.0 g, 5.46 mmol) dropwise in dry acetonitrile (50 mL). After addition the reaction mixture was heated to 70 °C for 1–2 h until complete by TLC (92:8:0.1 $CH_2Cl_2/CH_3OH/NH_4OH$). The solvent was removed in vacuo and the product purified by column chromatography on silica gel (70–230 mesh) eluting with 95:5 CH_2Cl_2/CH_3OH. The fractions containing product were pooled together and evaporated, and the product recrystallized from 2-propanol to afford 1.6 g (88%). A similar procedure was used for the preparation of the remaining acylureas.

General Procedure for Preparation of Hydrochloride Salts. To an ice-cold solution of the benzoylurea (5 mmol) in methanol (20 mL) and dichloromethane (10 mL) was added 1.0 M HCl in diethyl ether (5 mL) with stirring. After the addition the volume was reduced by two-thirds and triturated with diethyl ether to provide the salt as a solid which was recrystallized from 2-propanol.

Biology. (a) Mouse Assay. Male Swiss Webster mice (Crl:CFW SW BR, Charles River Breeding Lab, Wilmington, MA) of 17–20 gm weight range were used for the infection. Reservoirs of *N. dubius* and *H. nana* were maintained in nonmedicated male Swiss Webster mice for up to 22 days (*H. nana*) or 2 months (*N. dubius*).

L_3 larvae of *N. dubius* and eggs of *H. nana* were obtained from the non-medicated passage mice as follows: For *H. nana*, tape worms were removed from the intestine the day before the infection and maintained at 4 °C. After grinding the worms on the day of the infection, eggs were collected and counted. For *N. dubius*, fecal pellets were placed on filter paper moistened with water and stored at room temperature for 5 days. At this time the L_3 larvae were collected from the edge of the filter paper by adding more water. The L_3 larvae were stored at 4 °C for up to 2 months and counted on the day of the infection. Animals were challenged by oral gavage with a mixture of 35–40 L_3 larvae of *N. dubius* and 5000 eggs of *H. nana* per mouse.

Table II. Anthelmintic Activity in Sheep

compd	*RS* or *S*	dose, mg/kg po	% reduction vs control[a] Hc	Oc	Tc
14	*RS*	30	98	93	99
14	*RS*	30	100[b]	55[c]	92[b]
14	*RS*	15	92	75	100
13	*S*	15	86	74	98
13	*S*	10	90	64	96
13	*S*	5	74	56	75
13·HCl	*S*	16.4	78	82	99
13·HCl	*S*	10.9	64	52	93
19	*S*	10	59	29	40
19	*S*	5	27	33	15
levamisole HCl	*S*	7.5	100	97	100

[a] Untreated. Hc = *H. contortus*, Oc = *O. circumcincta*, Tc = *T. colubriformis*. [b] Benzimidazole-resistant strain. [c] Benzimidazole-, levamisole-, and morantel-resistant strain.

Starting 24 h after infection, groups of four mice were treated for 18 days ad lib with compound mixed in the food at concentrations ranging from 31 to 1000 ppm. The medicated food was prepared by mixing powdered Purina Lab Chow with compound for 18 h with a reversible rotary feed mixer. Levamisole at 62 ppm or oxfendazole at 31 and 125 ppm served as positive controls.

On day 19 after infection, mice were sacrificed and examined for parasite burden in the intestine. The entire small intestine was removed, placed between two large glass plates (3 × 5 in.) and viewed under a dissecting microscope. The number of *N. dubius* and *H. nana* were counted in the duodenum and ileum, respectively. The percent reduction in worm burden was calculated as follows:

$$\frac{\text{mean no. worms (control)} - \text{mean no. worms (treated)}}{\text{mean no. worms (control)}} \times 100$$

The results are shown in Table I.

(b) Sheep Assay. Merino ewes and wethers, ca. 12–15 months of age and weighing between 25 and 35 kg, were maintained at the Australian Research Unit field facility, Syntex Research, New South Wales, Australia. The screening trials for this set of compounds were run from January 1989 to February 1990. The animals were fed an ad lib ration of lucerne and oaten chaff and allowed free access to water. For each compound challenge, benzimidazole-susceptible L_3 larvae of *H. contortus* (6000), *O. circumcincta* (8000), and *T. colubriformis* (8000) were administered via intraruminal injection (day 0). On the basis of faecal nematode egg counts at day 27, the animals were ranked, weighed, and allocated to treatment groups (n = 4–7).

Compounds selected from the mouse assay for further in vivo testing in sheep were suspended in a 0.5% sodium (carboxymethyl)cellulose saline (0.9% NaCl) solution. The required dose was delivered via intraruminal injection (day 28). Each test compound was tested in a separate assay. In every assay, a control group received no treatment.

On day 35 after infection, the sheep were sacrificed, the abomasum and small intestine were removed, and the total number of adult *H. contortus*, *O. circumcincta*, and *T. colubriformis* were determined. The percent reduction in worm burden per treatment group for each worm species was calculated as in the mouse assay. The results are shown in Table II.

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