Anthelmintic Profile of the Cyclodepsipeptide PF1022A in *In Vitro* and *In Vivo* Models

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A novel cyclodepsipeptide of fungal origin, PF1022A, recently was reported to have anthelmintic activity. To supplement published reports and determine potential utility of PF1022A as a ruminant anthelmintic, the compound was examined in *in vitro* and *in vivo* models. Assays used measured motility of *Haemonchus contortus* (intrinsic drug potency), ATP levels (parasite death), and activity against *H. contortus*, *Ostertagia ostertagi*, and *Trichostrongylus colubriformis* in the jird (spectrum, potency, and efficacy by various routes). The potency of PF1022A in reducing motility is greater than commercial anthelmintics. Examination of ATP levels in PF1022A-paralyzed *H. contortus* indicates that worms are not killed, suggesting the compound acts as a neurotoxin in nematodes. In the jird, PF1022A has activity orally against each of the parasites studied and at doses comparable to all commercial anthelmintics, except the macrocyclic lactones which are more potent. Unfortunately, for some nematode species, parenteral delivery is ineffective at realistic doses.

A novel cyclodepsipeptide of fungal origin, PF1022A, was recently reported to have anthelmintic activity by SASAKI *et al.*[1]. This activity was discovered in a screen where 2 mg/kg of the compound cleared >91% of *Ascaridia galli* from chickens. TERADA[2] and TERADA *et al.*[3] have noted PF1022A is effective orally against *Toxocara canis* and *Toxocara cati* in dogs at 0.2 mg/kg, orally in cattle (dose not identified) and intravenously (neither dose nor host identified) against *Haemonchus contortus*, and against *Ostertagia ostertagi* in cattle (neither route nor dose identified), while motility of *Heterakis spumosa* is inhibited *in vitro* within 2 hours after treatment at 10^{-7} g/ml. At 1 g/kg intraperitoneally or 2 g/kg orally, SASAKI *et al.*[1] reported PF1022A produced no signs of acute toxicity in mice. To supplement these results and determine the potential utility of PF1022A as a ruminant anthelmintic, we examined the compound in a variety of *in vitro* and *in vivo* models. Results of these studies are reported herein.

Materials and Methods

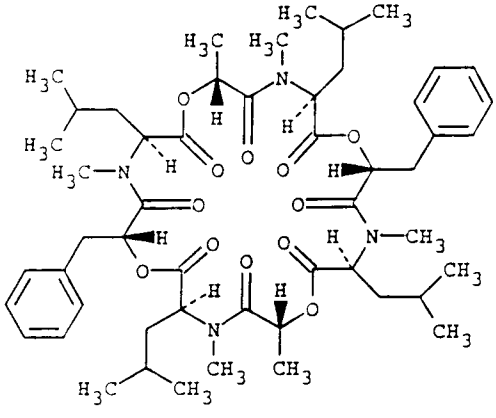
Compound

PF1022A (Fig. 1) was produced synthetically at The Upjohn Company using the methods described by DUTTON and NELSON[4]. The standards used in these studies were levamisole (Sigma Chemical Co.), albendazole (Smith Kline Beecham), and ivermectin (Merck & Co.).

Motility Assay

In vitro motility assays using adult female *H. contortus* were conducted to determine the intrinsic potency of PF1022A relative to broad-spectrum anthelmintics. Details regarding collection of parasites, other materials used, and the operation of the automated micromotility recording system were described previously by GEARY *et*

Fig. 1. Structure of PF1022A.



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al.[5]. The vehicle used in this and the other *in vitro* assays discussed in this report was DMSO; the vehicle concentration was 0.1%. Briefly, culture tubes containing 5 adult female *H. contortus* and various concentrations of PF1022A or anthelmintic standard were loaded into a test tube rack and maintained at 37°C. At specified intervals, the tubes were individually transferred by a Zymark robot into a vortexer and, following 5 seconds of vigorous shaking, transferred to a 4-channel micromotility meter (B&P Instruments, E. Lansing, MI) for recording. The recording apparatus consisted of an infrared light emitting diode and a detector diode positioned to record scattered photons, the intensity of which depends on the frequency of *H. contortus* movements in the culture tubes. The analog signal from the detector was digitized and transferred electronically to an IBM-AT computer for processing. The signal from heat-killed controls (K, 20~30 counts/minute), which represents random vibration, was automatically subtracted from signals in vehicle control (C) and drug-treated (T) groups. At the completion of each study, measurements were standardized by converting the vibration-corrected signal from treatment groups to the percent of control, such that motility values used in subsequent analyses were based on the following equation:

$$\text{Motility (\% of control)} = \frac{T-K}{C-K} \cdot 100$$

Individual data points are based on at least 5 separate experiments (4 culture tubes/treatment group/experiment).

ATP Assay

To determine if the paralyzing actions of PF1022A are associated with a reduction in the energy charge of worm tissue, ATP levels were measured in *H. contortus* at termination (24 hours) of motility recordings. The incubates tested were those exposed to 0.1 μM PF1022A, a concentration sufficient to induce complete paralysis of *H. contortus* within 1 hour. Details regarding this assay were described previously by GEARY *et al.*[5].

Jird Assays

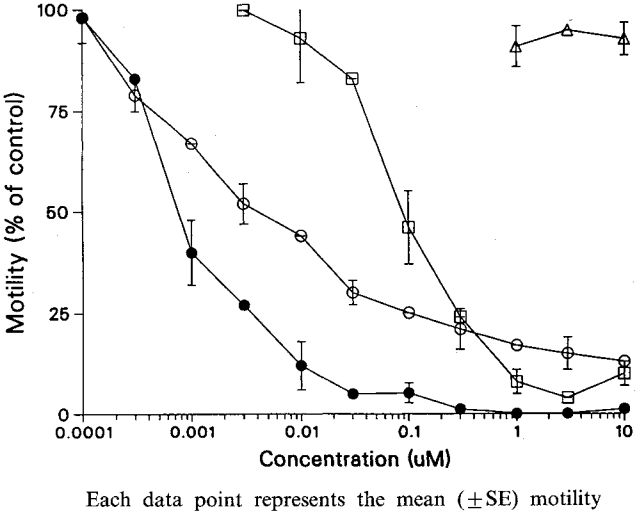
To assess the potential spectrum of PF1022A against ruminant nematodes, activity against 3 representative species (*H. contortus*, *Trichostrongylus colubriformis*, and *O. ostertagi*) was determined in a jird model. Evaluation against a mixed infection of *H. contortus* and *T. colubriformis* was done as outlined by CONDER *et al.*[6], except that *T. colubriformis* was not exsheathed prior to inoculation. In the case of *O. ostertagi*, similar methods to those of CONDER *et al.*[6] were used, with the following changes. The parasite was exsheathed by exposure to Earle's Balanced Salt Solution + CO_2 for 18 hours; each jird was inoculated with 250 exsheathed infective larvae; treatment was on day 6 postinoculation (PI) and necropsy on day 8 PI. Activity against each of the 3 nematode species was determined orally (PO), intraperitoneally (IP), subcutaneously (SC), intramuscularly (IM), and percutaneously (PC). In each case, the drug was given in DMSO (17%)/vehicle #98 (83%), except for PC where the vehicle was DMSO. Each experiment was repeated at least twice.

Results and Discussion

The intrinsic activity of PF1022A against *H. contortus* in the motility assay is dramatic. Compared to compounds (albendazole, ivermectin, and levamisole) representing the 3 classes of commercial broad-spectrum anthelmintics, PF1022A is the most potent (Fig. 2). It is 3~10× more potent than ivermectin (based on the nominal concentrations of drugs in these incubates) and produces complete paralysis of *H. contortus* at 0.1 μM. To determine whether the complete paralysis observed by motility analysis is indicative of parasite death, ATP levels in PF1022A-treated worms were determined and compared to those of standard-treated, vehicle-treated, and heat-killed worms. Figure 3 shows that, although paralyzed, PF1022A-treated *H. contortus* contain ATP at levels comparable to worms treated with vehicle (DMSO) only, while heat-killed parasites exhibit little ATP. These data suggest PF1022A acts as a neurotoxin in nematodes.

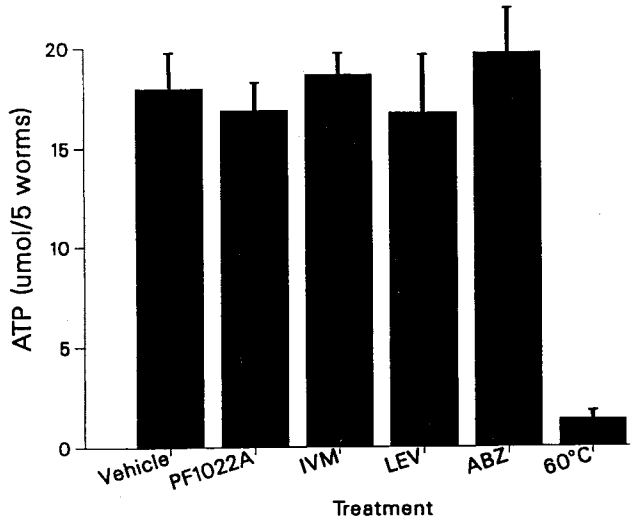
In the jird, PF1022A has excellent activity against each of the 3 nematode species examined, when administered PO. Table 1 shows the oral 95% effective dose for each parasite in the jird for PF1022A compared to representative compounds from each of the 3 classes of broad-

Fig. 2. Concentration-dependent effects of PF1022A (●), ivermectin (○), levamisole (□), and albendazole (△) on motility of adult female *Haemonchus contortus* following 24 hour incubations.



Each data point represents the mean (±SE) motility value, normalized to percent of DMSO controls, from at least 5 separate experiments.

Fig. 3. ATP levels in *Haemonchus contortus* incubates exposed for 24 hours to PF1022A or selected broad-spectrum anthelmintics.



The 60°C treatment represents heat-killed worms. Concentrations tested were: PF1022A (0.1 μM), ivermectin (IVM, 0.1 μM), levamisole (LEV, 10 μM), albendazole (ABZ, 10 μM). Each data point represents the mean (±SE) ATP level in at least 4 separate incubates containing 5 adult female *H. contortus*.

Table 1. The minimum 95% effective oral dose (mg/jird*) for PF1022A and 3 commercial anthelmintics against 3 ruminant nematodes in the jird.

Compound	*Haemonchus contortus*	*Ostertagia ostertagi*	*Trichostrongylus colubriformis*
PF1022A	0.11	0.33	0.11
Ivermectin	0.005	0.02	0.0025
Albendazole	0.075	2.5	0.125
Levamisole	0.4	0.4	0.075

* 1 mg/jird ≅ 25 mg/kg.

Fig. 4. Mean percentage clearance (3~8 experiments per data point) of *Haemonchus contortus* from jirds 3 days following treatment with PF1022A by various routes.

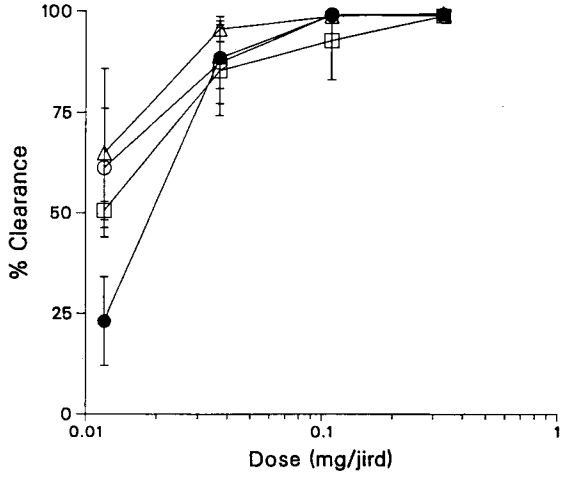


Fig. 5. Mean percentage clearance of *Haemonchus contortus* over time (2~3 experiments per data point) from jirds following treatment with PF1022A at 0.11 mg/jird (~2.75 mg/kg) by various routes.

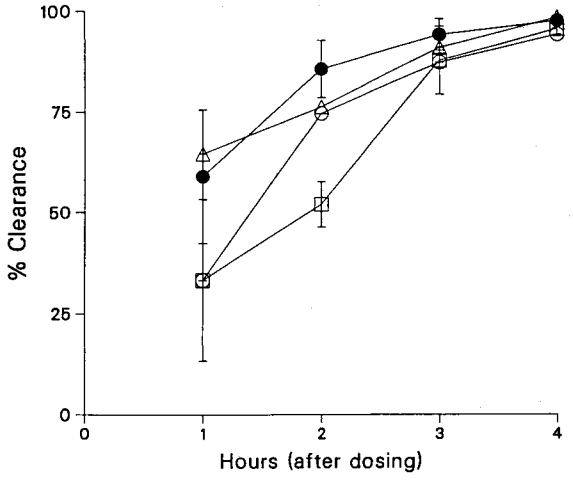


Fig. 6. Mean percentage clearance (3~7 experiments per data point) of *Trichostrongylus colubriformis* from jirds 3 days following treatment with PF1022A by various routes.

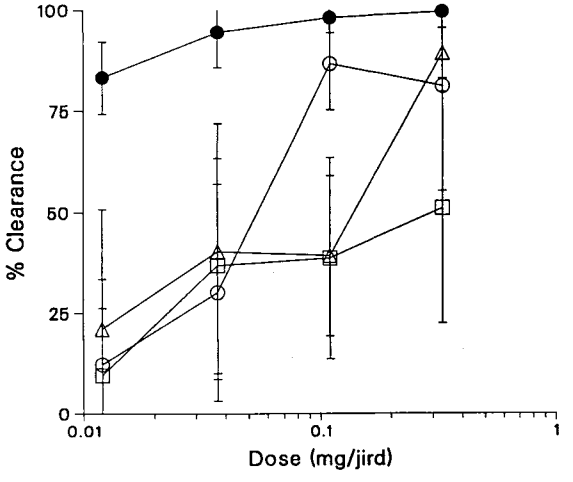
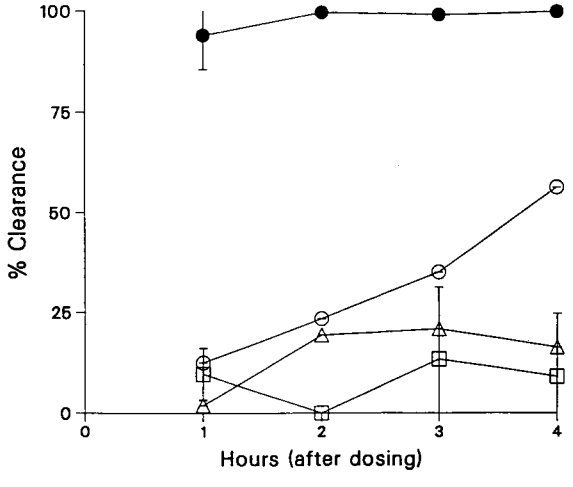


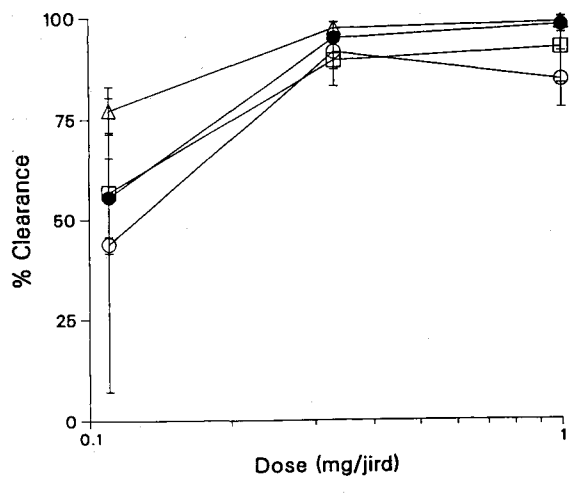
Fig. 7. Mean percentage clearance of *Trichostrongylus colubriformis* over time (2~3 experiments per data point) from jirds following treatment with PF1022A at 0.11 mg/jird (~2.75 mg/kg) by various routes.



spectrum anthelmintics. In this model, unlike the *in vitro* motility assay, PF1022A is not the most potent anthelmintic. Ivermectin is dramatically more potent than the other drugs shown in Table 1 against each of the 3 nematodes, while PF1022A and the other 3 classes all have comparable potency against each parasite, although rank order of potency varies from worm to worm among the drugs. Since the jird model has been shown to be predictive for utility in the ruminant by CONDER *et al.*[6,7] and PF1022A appears to show good activity in the model against each of the 3 important parasites used, it is likely that PF1022A will have useful activity in ruminants.

Unfortunately, PF1022A, delivered by routes other than oral, does not appear to be effective at realistic doses against some nematode species. Although there is no apparent difference in potency at effective doses against *H. contortus*, either in time to clear or percentage clearance 3 days post-treatment, when the drug is given PO, IP, SC, IM (Figs. 4 and 5), its potency is markedly reduced for *T. colubriformis* when administered IP, SC, or IM compared to PO (Figs. 6 and 7). Even at the highest dose examined (2 mg/jird≅50 mg/kg), 95% clearance is not achieved for *T. colubriformis* by parenteral routes (data not shown), while 0.11 mg/jird (~2.75 mg/kg) is effective PO. This difference in parenteral efficacy between *H. contortus* and *T. colubriformis* might be explained by the feeding habits of the 2 worms. *Haemonchus contortus* is a voracious blood feeder and may gain access to parenterally delivered drug during feeding. In contrast, *T. colubriformis* is a detritus feeder and, as such, probably comes in contact only with drug that is present in the lumen of the gut. Apparently, PF1022A fails to achieve a sufficient luminal level to be efficacious against this parasite when administered parenterally. Interestingly, *O. ostertagi*, which has feeding habits between the other 2 nematode species, exhibits sensitivity to PF1022A by some but not all parenteral routes. Figure 8 shows that although IM delivered drug provides comparable results to that given PO for *O. ostertagi* (*i.e.* ≥95% clearance at 0.33 mg/jird≅8.25 mg/kg), PF1022A given IP or SC is <95% effective at 1 mg/jird (25 mg/kg). Percutaneous delivery was ~95% effective for *H. contortus* only at the highest dose examined (2 mg/jird), while this dose was ineffective PC against *O. ostertagi* and *T. colubriformis*. Hence, PF1022A appears to have limited delivery options.

Fig. 8. Mean percentage clearance (2 experiments per data point) of *Ostertagia ostertagi* from jirds following oral treatment with PF1022A.



In summary, PF1022A has potent activity against a spectrum of ruminant nematodes. Unfortunately, it appears to lack useful parenteral activity against some species.

Acknowledgment

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