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Discovery of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives active against *Haemonchus contortus* and *Ctenocephalides felis* (cat flea)

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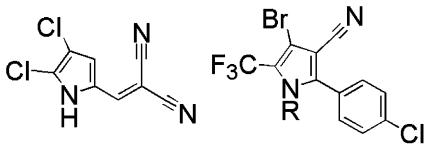
Abstract—A series of 2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives were synthesized and evaluated for in vitro activity against the endoparasite *Haemonchus contortus* and the ectoparasite *Ctenocephalides felis*. Some compounds had significant in vitro activity against these parasites.

The development of the macrocyclic lactone class of parasiticides, exemplified by ivermectin, was a landmark in the control of endoparasites, especially nematodes, in human and animal health.[1] The success of this class of potent endoparasiticides is reflected by the subsequent failure of the animal healthcare industry to introduce any new class of endoparasiticidal drugs over the past 25 years, despite growing reports of the development of parasite resistance to the macrocyclic lactones and to other classes of parasiticide drugs in current use. In particular, multi-drug endoparasite resistance threatens the viability of small-ruminant production in many tropical regions and also in temperate areas of southeast USA, Australia, and New Zealand.[2]

We commenced research aimed at the discovery of new drugs to control endoparasites, based upon high-throughput screening of compounds in the commercial NemaTox *Haemonchus contortus* (McMaster strain) larval development assay.[3,4]

From this exercise we obtained the hit compound, (4,5-dichloro-1*H*-pyrrol-2-yl)methylenemalononitrile **1** (*H. contortus* LD_{99} = 1.8 μM) (Fig. 1). Significantly, compound **1** maintained activity against resistant strains of *H. contortus*: LD_{99} = 2.2 μM for the benzimidazole- and levamisole-resistant Lawes strain and LD_{99} = 2.2 μM for the avermectin-resistant CAVR strain. Furthermore, compound **1** was active against the McMaster strains of *Ostertagia circumcinta*, LD_{99} = 2.2 μM, and *Trichostrongylus colubriformis*, LD_{99} = 9.0 μM. Compound **1** was inactive in an ectoparasite *Ctenocephalides felis* (cat flea) assay.[5]

Compound **1** is a potential Michael acceptor and therefore a potential non-specific general toxin arising from



1 **2** R = $EtOCH_2$ (Chlorfenapyr)
3 R = H

Figure 1.

Keywords: (Z)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile; Parasiticide; *Haemonchus contortus*; *Ctenocephalides felis* (cat flea).
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covalent binding to cellular nucleophiles. Compound **1** is not universally toxic. For example, compound **1** was active against the bacteria *Bacillus subtilis* (LD_{99} = 1.7 μM) and the murine NS-1 myeloma cell line (LD_{99} = 13 μM) but inactive against the yeast *Saccharomyces cerevisiae*. This selective toxicity indicated that compounds of this type were suitable for further investigation.[6]

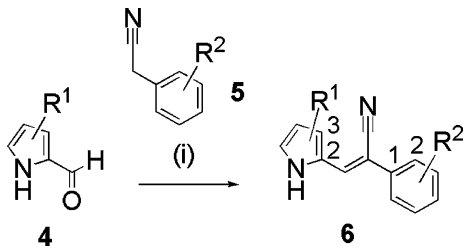
We hypothesized that compound **1** might act as an uncoupler of oxidative phosphorylation, because of structural similarity to the insecticide chlorfenapyr **2** (Pirate™). Chlorfenapyr is a prodrug whose active metabolite **3** acts as an uncoupler of oxidative phosphorylation in mitochondria.[7]

To probe SAR associated with compound **1**, we prepared a set of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitriles **6** from benzyltrimethylammonium hydroxide-catalyzed Knoevenagel condensation reactions of various 1*H*-pyrrole-2-carboxaldehydes **4** with phenylacetonitrile derivatives **5** performed in either ethanol or water (Scheme 1 and Table 1).[8–12]

With particularly hydrophobic reactants, the reaction was more conveniently catalyzed using 18-crown-6 and potassium hydroxide in toluene. For some sterically hindered *ortho*-substituted phenylacetonitrile derivatives, it was necessary to conduct the reaction at elevated pressure in a sealed glass tube.

A number of these compounds which contained electron withdrawing substituents at either the pyrrole ring or the phenyl ring or at both rings displayed excellent activity against *H. contortus* (Table 1). Many compounds showed levels of activity comparable to the commercial endoparasiticidal drugs levamisole and closantel. Significantly, some of these compounds were also active against *C. felis*, indicating that the 3-(1*H*-pyrrol-2-yl)acrylonitrile template had the potential to deliver valuable broad-spectrum endectoparasiticidal activity. In particular, the 4-chlorophenyl derivative **26** gave an LD_{99} = 1.8 μM in the nematode assay, 95% mortality in a rapid cat flea mortality assay, and an LC_{50} = 0.17 μg/cm^2 for *C. felis*.

We observed that calculated pK_a values for **1** and **26** (10.6 and 15.0, respectively) fall outside the range 7–8 reported as being necessary for uncoupling of oxidative phosphorylation, which indicates that these



Scheme 1. Reagents: (i) $PhCH_2NMe_3(OH)$, $EtOH/H_2O$ or 18-crown-6, KOH, $PhCH_3$.

compounds, unlike chlorfenapyr, do not act by this mode of action.[7,13] Consistent with this view, we also observed that electron withdrawing substituents at the pyrrole ring decreased ectoparasiticide activity: for example, the 4,5-dichloropyrrole derivative **40** (calculated pK_a = 11.8) is much less active against *C. felis* than the corresponding unsubstituted pyrrole analog **26**.

At the 4 position of the phenyl ring of the 3-(1*H*-pyrrol-2-yl)acrylonitrile template small substituents such as H, halogen, Me, Et, and CF_3 afforded the best activity against *H. contortus*. Best activity against *C. felis* was observed for compounds containing H, F, Cl, *i*-Pr or *t*-Bu as a substituent at the 4 position of the phenyl ring.

At the 3 position of the phenyl ring of the 3-(1*H*-pyrrol-2-yl)acrylonitrile template the substituents could be ranked as follows for *C. felis* activity: F ≫ CF_3 > Me > Cl > Br > PhO, but only the 3-fluorophenyl derivative **16** exhibited activity comparable to the most active of the 4-substituted compounds. With respect to activity against *H. contortus* 3-phenyl-substituted compounds were generally less active compared to the corresponding 4-substituted derivative.

Ctenocephalides felis mortality for compounds containing a substituent at the 2 position of the phenyl ring was ranked: Me > H > F ≫ Br > Cl > CF_3 > CN > OMe > Ph. The *ortho*-methyl-substituted compound **12** was the most active ectoparasiticide discovered in this work. With respect to activity against *H. contortus*, 2-phenyl-substituted compounds were generally less active compared to the corresponding 4-substituted derivative.

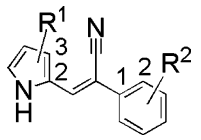
Di-substitution by halogen at the phenyl ring of the 3-(1*H*-pyrrol-2-yl)acrylonitrile template generally led to diminished activity against both *H. contortus* and *C. felis*.

In an attempt to improve the parasiticidal activity of compound **26**, we prepared a small set of derivatives in which the pyrrole nitrogen was capped (Table 2). Compounds **53**, **54**, **56**, and **59** were prepared by reaction of the appropriate capped 1*H*-pyrrole-2-carboxaldehyde with 4-chlorophenylacetonitrile whereas compounds **55**, **57**, **58**, **60**, **61**, **62**, **63**, and **64** were prepared from the reaction of **26** with the appropriate electrophilic capping reagents.[12,14]

With the exception of compound **55** all of these capped compounds were significantly less active than the uncapped compound **26** against *H. contortus*. None of the capped compounds showed significant activity against *C. felis*.

To summarize, unsubstituted (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (compound **7**) retained the best balance of endo- and ectoparasiticide activity (*H. contortus* LD_{99} = 2.4 μM; *C. felis* LC_{50} = 0.26 μg/cm^2,

Table 1. Activity of compounds **7**–**52** against *H contortus* (*H.c.*) and *C. felis* (*C.f.*)



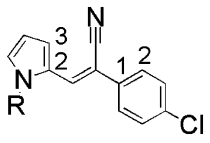
Compound	R^1	R^2	*H.c.* LD_{99}[a] (μM)	*C.f.* % Kill[a,b]	*C.f.* LC_{50}[a] (μg/cm^2)
7	H	H	2.4	98	0.26
8	H	2-F	12	92	0.59
9	H	2-Cl	12	19	
10	H	2-Br	12	30	
11	H	2-CF_3		14	
12	H	2-Me	18	100	0.08
13	H	2-Ph		2	
14	H	2-OMe		9	
15	H	2-CN	>68	11	
16	H	3-F	8	78	0.78
17	4-Br	3-F	38	18	
18	H	3-Cl	3.3	28	
19	H	3-Br	3.4	24	
20	H	3-CF_3	3.6	56	9.9
21	4-Br	3-CF_3	9.5		
22	H	3-Me	6.0	42	
23	H	3-OPh	11	10	
24	H	4-F	2.0	78	0.30
25	4-Br	4-F	17	15	
26	H	4-Cl	1.8	95[c]	0.17
27	4-Br	4-Cl	1.5	23	
28	H	4-Br	0.84	42	
39	H	4-I	2.2	14	
30	H	4-CF_3	3.1	20	
31	4-Br	4-CF_3	>44	15	
32	H	4-Me	4.5	69	
33	H	4-Et	4.0	58	
34	H	4-OMe	6.7	31	
35	H	4-CN	46	44	
36	H	4-NO_2	>63	6	
37	H	4-*i*-Pr		100	1.32
38	H	4-*t*-Bu	13	87	0.48
39	H	4-Ph	28	46	
40	4,5-diCl	4-Cl	10	15	
41	H	2,4-diCl	11	8	
42	H	3,4-diCl	2.9	41	
43	4-Br	3,4-diCl	7.3	19	
44	H	2-Cl, 4-F	12	30	
45	H	3-Cl, 4-F	5.1	5	
46	H	2-Cl, 6-F	>61	7	
47	H	2-F, 4-Cl	10	5	
48	H	2,4-diF	11	5	
49	H	2,6-diF	52	17	
50	H	3,4-diF	7.6	17	
51	H	3,5-diCF_3	>45	27	
52	H	2,4-diMe		44	
Levamisole			0.66–2.0		
Closantel			9.4–18		
Ivermectin			0.0057–0.031		
Fipronil					0.018

[a] No entry indicates that the compound was not assayed.
[b] Measured at 24 h.
[c] Measured at 8 h.

respectively), whereas (*Z*)-2-(3-methylphenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (compound **12**) displayed the best ectoparasiticide activity (*C. felis* LC_{50} = 0.08 μg/cm^2).

There is significant potential for further exploration of parasiticide activity associated with structural modification to the (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile template discovered from our research.

Table 2. Activity of compounds **53**–**64** against *H. contortus* (*H.c.*) and *C. felis* (*C.f.*)



Compound	R	*H.c.* LD_{99} (μM)	*C.f.* % Mortality[a]
26	H	1.8	95[b]
53	Me	7.8	13[b]
54	CH_2OEt	19	6[b]
55	Propanoyl	1.3	12
56	Benzyl	44	3
57	$Et_2NC(O)$	11	8
58	*tert*-Butoxycarbonyl	11	9
59	3-Methyl-2-butenyl	>51	0
60	Benzoyl	3.8	6
61	Tosyl	31	0
62	$Me_2NC(S)$	32	19
63	MeOC(O)	4.4	16
64	Benzyloxycarbonyl	15	20

[a] Measured at 24 h.
[b] Measured at 8 h.

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5. (a) *Ctenocephalides felis* assays were conducted by the Centre for Entomological Research and Insecticide Technology, UNSW, Randwick, NSW, Australia; (b) In vitro single dose *C. felis* assay: the test compound was dissolved in acetone (0.5 mL) and applied to the base of four 100 mL flasks at a concentration of 1.26 μg/cm^2. Flasks were left to dry for 24 h before flea exposure. Controls were prepared in the same manner, except that no test compound was dissolved in the acetone. Fifteen adult fleas, aged between 3 and 7 days postemergence, were then introduced into each flask. The top of the flasks was covered in Parafilm™ and small holes were made to allow gas exchange. The treatment flasks were held at 25 ± 1 °C and 75 ± 5% humidity for 24 h. Dead fleas in each flask were counted and the pooled data were converted to percentages; (c) In vitro *C. felis* LC_{50} measurement: six concentrations of test compound were obtained from serial dilution of an acetone solution of the compound. For each test compound this set of doses covered a range that produced very low to very high flea mortality; this range was determined from a pilot study. Mortality resulting from the treatments was recorded at 24 h. Pooled mortality data were subjected to probit analysis to obtain concentration response data (LC_{50}) (Finney, D. J. *Probit Analysis*, 3rd ed., Cambridge Univ. Press, London, 1971).
6. Subsequently, in separate experiments we found no ^{1}H NMR spectroscopic evidence that compound **26** acts as a Michael acceptor when treated with excess ethanethiol in $CDCl_3$ or excess sodium methanethiolate in CD_3OD at room temperature for 24 h, consistent with the view that compounds **6** would not act as general toxins. We thank a referee for suggesting the use of thiol reagents to probe the propensity of compounds of this type to act as a partner in covalent binding to cellular nucleophiles. Further in vitro studies with mammalian cells will be required to unequivocally clarify the mammalian safety of compounds derived from the (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile template.
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11. Synthesis of 2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile compounds, typical procedures:[10] Compound **12**: a thick-walled tube was charged with 1*H*-pyrrole-2-carboxaldehyde (2.0 g, 21.0 mmol), 2-methylphenylacetonitrile (2.5 g, 18.9 mmol), EtOH (75 mL), and 40% aqueous benzyltrimethylammonium hydroxide (4.0 mL, 3.3 mmol). The tube was sealed and heated to 90 °C for 4 days. The solvent was evaporated and the residue subjected to chromatography on silica gel eluting with CH_2Cl_2 /light petroleum (20:80) to afford compound **12** as a yellow solid (1.2 g, 30% yield), mp 87–89 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.81 (br s, 1H), 7.18–7.30 (m, 4H), 7.07 (m, 1H), 7.03 (s, 1H), 6.63 (m, 1H), 6.34 (m, 1H), 2.48 (s, 3H). MS (EI) 208 (M^+). Compound **26**: a suspension of 1-*H*-pyrrole-2-carboxaldehyde (3.3 g, 34.7 mmol) and 4-chlorophenylacetonitrile (5.5 g, 33.0 mmol, 0.95 equiv) in H_2O (50 mL) was heated to 50 °C. After all the solid material had melted, the vigorously stirred mixture was treated with 40% aqueous benzyltrimethylammonium hydroxide (14 mL, 5.6 mmol). Stirring was continued for 5 h at 50 °C, breaking up lumps when necessary. The still warm suspension was filtered through a sintered glass funnel and the yellow precipitate was washed with warm H_2O and dried to afford compound **26** (6.63 g, 88% yield), mp 116–118 °C, lit mp 122–123 °C.[8]
12. All synthetic intermediates and final products were characterized by ^{1}H NMR and MS.
13. pK_a values were calculated using SPARC Aug2003 server <http://ibmlc2.chem.uga.edu/sparc/>. The calculated pK_a values for pyrrole compounds with known pK_a values differed consistently by about half a log unit. For example, the calculated pK_a for compound **3** is 8.19, whereas the measured pK_a is reported to be 7.6.[7]
14. Synthesis of capped pyrrole derivatives, typical procedures:[10] Compound **54**: to a solution of 4-chlorophenylacetonitrile (500 mg, 3.30 mmol), 1-ethoxymethyl-1*H*-pyrrole-2-carboxaldehyde (520 mg, 3.39 mmol), and 18-crown-6 (87 mg, 0.33 mmol) in toluene (30 mL) was added KOH (185 mg, 3.30 mmol) and the mixture was heated to 80 °C for 3 h, then stirred at room temperature overnight. The solution was filtered through a small plug of silica and concentrated to afford compound **54** as an oil (705 mg, 73% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.56 (m, 1H), 7.55 (d, *J* 8.8 Hz, 2H), 7.38 (d, *J* 8.8 Hz, 2H), 6.93 (m, 1H), 6.33 (m, 1H), 5.39 (s, 2H), 3.44 (q, *J* 6.9 Hz, 1H), 1.18 (t, *J* 6.9 Hz, 1H). MS (APCI+) 287.2 (M+1). Compound **55**: to a stirred solution of compound **26** (600 mg, 2.62 mmol), Et_3N (320 mg), and 4-dimethylaminopyridine (32 mg) in CH_2Cl_2 (20 mL) was added

propionic anhydride (375 mg). The mixture was stirred at room temperature for 7 h. Et_3N (320 mg) and propionic anhydride (375 mg) were added and the mixture was stirred at room temperature for 48 h. The reaction mixture was poured into ether (200 mL) and the ether was washed with 10% aqueous citric acid solution (50 mL), H_2O (2×50 mL), and saturated brine (50 mL). The ether layer was collected, dried over Mg_2SO_4, filtered, and evaporated. The residue was recrystallized from ether/light petroleum to afford compound **55** as yellow needles (611 mg, 82% yield), mp 106–108 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.59 (d, J 8.8 Hz, 2H), 7.47 (d, J 4.4 Hz, 1H), 7.38 (d, J 8.8 Hz, 2H), 7.32 (dd, J 3.7 and 1.5 Hz, 1H), 6.43 (t, J 3.7 Hz, 1H), 2.97 (q, J 7.3 Hz, 2H), 1.31 (t, J 7.3 Hz, 3H). MS (EI) 284 (M^+). Compound **59**: a solution of **25** (200 mg, 0.875 mmol) in dry acetone (35 mL) was treated with K_2CO_3 (121 mg) followed by 1-bromo-3-methyl-2-butene (111 μL, 0.962 mmol). The mixture was heated to a gentle reflux for 20 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was chromatographed on silica gel eluting with EtOAc/light petroleum (1:1) to afford compound **59** as a yellow oil (85 mg). ^{1}H NMR (200 Hz, $CDCl_3$) δ 7.47–7.50 (m, 3H), 7.32–7.41 (m, 2H), 6.88 (s, 1H), 6.31 (s, 1H), 5.30 (t, J 6.2 Hz, 1H), 4.62 (s, 1H), 4.60 (s, 1H), 1.79 (s, 3H), 1.78 (s, 3H). MS (EI) 296 (M^+).