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Novel thiophenes and analogues with anthelmintic activity against *Haemonchus contortus*

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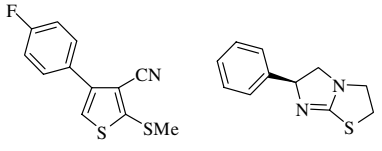
Abstract—A new series of analogues of 4-(4-fluorophenyl)-2-methylthio-thiophene-3-carbonitrile (**1**) were synthesized and evaluated for their in vitro and in vivo anthelmintic activity against *Haemonchus contortus*.

Discovery of structurally distinct anthelmintics with novel mechanisms of action is critical to address the challenges associated with the control of helminth infections in certain livestock sectors. The development of resistance in small ruminants (sheep) in Australia and southern Latin America has eroded the utility of most available anthelmintics, and is a constant threat to further reduce the options for treatment.[1,2] In the course of our search for novel anthelmintics, 4-(4-fluorophenyl)-2-methylthio-thiophene-3-carbonitrile (**1**)[3] (Fig. 1) was discovered as an initial lead toward in vitro anthelmintic activity against *Haemonchus contortus* (*H. contortus*) using a larval mobility assay (LMA).[4]

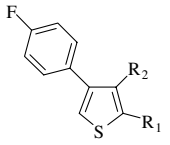
Thiophene **1** exhibited potent in vitro anthelmintic activity against *H. contortus* after 24 h of incubation, although was considerably less potent after only 2 h of incubation (Table 1). We observed that EC_{50} values



1 Levamisole (**2**)

Figure 1. Thiophene **1** and levamisole (**2**).

Table 1. In vitro anthelmintic activity of analogues of **1** with different substituents at the 2- and 3-positions of the thiophene ring



Compound	R_1	R_2	LMA 24 h EC_{50} (μM)	LMA 2 h EC_{50} (μM)
2			15	18
1	SMe	CN	28	>200
19	SMe	H	68	>200
20	SMe	Br	17	>200
21	SMe	NO_2	NA	—
23	SEt	CN	6	>200
26	H	CN	NA	—
28	NH_2	CN	NA	—
29	NHMe	CN	NA	—
30	SO_2Me	CN	NA	—
31	OEt	CN	NA	—
34	COMe	H	NA	—
35	Et	H	NA	—

NA: Less than 50% of inhibition of the motility at 100 ppm after 24 h of incubation using the LMA.

>200 μM were difficult to determine accurately, because of lack of correlation between the concentration and the potency and/or lack of reproducibility. Partial insolubility of the compounds at the higher concentrations of the assay might be the reason of the variability. Thiophene **1** did not show significant reduction in the number of *H. contortus* or *Trichostrongylus colubriformis*

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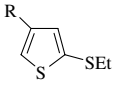
(*T. colubriformis*) nematodes in vivo using a gerbil model[5] at 100 mg/kg. Levamisole (**2**), a commercially available anthelmintic for control of *H. contortus,* showed potent in vitro anthelmintic activity using the LMA after 2 and 24 h of incubation (EC_{50} values of 15 and 18 μM after 2 and 24 h of incubation, respectively) and completely eliminates the number of nematodes (*H. contortus* and *T. colubriformis*) in the gerbil model at 10 mg/kg. We hypothesized that compounds with potent anthelmintic activity in short times of incubation in the LMA, might exhibit potent anthelmintic activity in vivo.

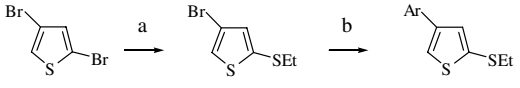
In an effort to improve the anthelmintic potency, several analogues of **1** with different substituents at the 2-, 3-, and 4-positions of the thiophene ring were prepared (Tables 1 and 2). 4-Aryl-2-ethylsulfanyl-thiophene analogues (**5**–**18**) were synthesized from 2,4-dibromo-thiophene (**3**) (Scheme 1). Lithiation of **3** with *n*-BuLi generated only the 2-lithio derivative, which on reaction with diethyldisulfide[6] gave **4**. Reaction of **4** with appropriate arylboronic acids by Suzuki coupling provided analogues **5**–**18**.[7] The 3-bromo-thiophene **20** was prepared from **19**[8] in two steps as shown in Scheme 2. Analogue **23** was synthesized from 2-ethylsulfanyl-thiophene **6** in three steps as indicated in Scheme 2.[9]

Table 2. In vitro anthelmintic activity of analogues of **5** with different substituents at the 4-position of the thiophene ring



Compound	R	LMA 24 h EC_{50} (μM)	LMA 2 h EC_{50} (μM)
2		15	18
1		28	>200
5	Phenyl	NA	—
6	*p*-F-phenyl	12	>200
7	*p*-NO_2-phenyl	15	193
9	*m*-CN-phenyl	189	>200
16	*m,p*-diF-phenyl	80	>200
17	2-Thienyl	161	>200
18	3-Pyridyl	33	46

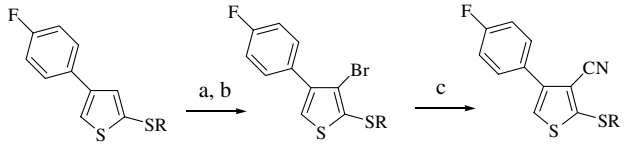
NA: Less than 50% of inhibition of the motility at 100 ppm after 24 h of incubation using the LMA.



3 **4**

	Ar		Ar
5	phenyl	**12**	*m*-NO_2-phenyl
6	*p*-F-phenyl	**13**	*o*-F-phenyl
7	*p*-NO_2-phenyl	**14**	*o*-CN-phenyl
8	*m*-F-phenyl	**15**	*o*-CF_3-phenyl
9	*m*-CN-phenyl	**16**	*m,p*-diF-phenyl
10	*m*-CH_3-phenyl	**17**	*2*-thienyl
11	*m*-CF_3-phenyl	**18**	*3*-pyridyl

Scheme 1. Reagents and conditions: (a) *n*-BuLi, EtSSEt, Et_2O, 83%; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O, 89% (**5**), 55% (**6**), 20% (**7**), 25% (**8**), 89% (**9**), 35% (**10**), 32% (**11**), 25% (**12**), 20% (**13**), 31% (**14**), 35% (**15**), 31% (**16**), 30% (**17**), 50% (**18**).



19 R=Me
6 R=Et

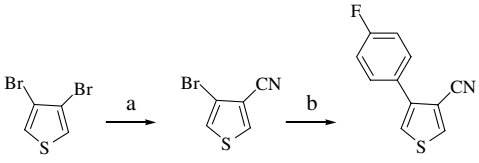
20 R=Me
22 R=Et

23 R=Et

Scheme 2. Reagents and conditions: (a) (i) Br_2, CH_2Cl_2; (ii) Zn, AcOH–H_2O–THF, 29% two steps (**20**); (b) (i) NBS, THF; (ii) Zn, AcOH–H_2O–THF, 31% two steps (**22**); (c) CuCN, NMP, 36%.

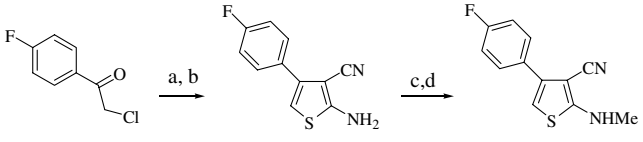
Thiophene **26** was prepared from 3,4-dibromo-thiophene (**24**) by treatment with copper(I) cyanide followed by Suzuki coupling with *p*-fluorophenylboronic acid (Scheme 3). The 2-amino-thiophene **28** was prepared from commercially available 2-chloro-1-(4-fluorophenyl)-ethanone (**27**) via formation of an ylidenemalonitrile intermediate followed by cyclization with sodium hydrosulfide in refluxing ethanol.[10] *N*-Monomethylation of **28** by reduction[11] of an intermediate alkyl imidate gave **29** (Scheme 4). Oxidation of **1** with *m*-chloro-perbenzoic acid provided 2-methanesulfonyl-thiophene **30**, which on reaction with sodium ethoxide yielded **31** (Scheme 5).

Selective bromination of 2-acetyl-thiophene (**32**) provided intermediate **33**,[12] which upon standard Suzuki



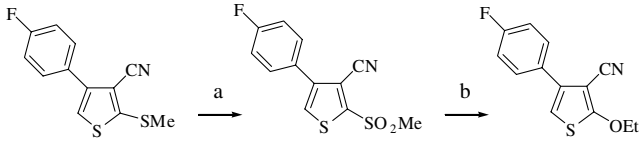
24 **25** **26**

Scheme 3. Reagents and conditions: (a) CuCN, NMP, 53%; (b) *p*-fluorophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O, 93%.



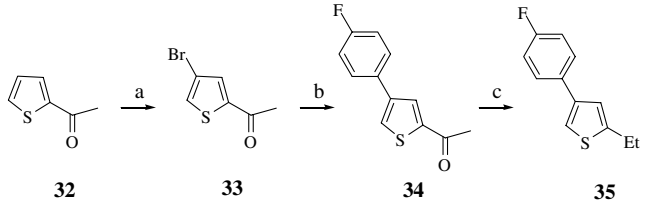
27 **28** **29**

Scheme 4. Reagents and conditions: (a) $CNCH_2CN$, NH_4OAc, benzene–AcOH, 40%; (b) NaSH, EtOH, 71%; (c) trimethyl orthoformate; (d) $NaBH_4$, EtOH, 78% (two steps).



1 **30** **31**

Scheme 5. Reagents and conditions: (a) MCPBA, CH_2Cl_2, 87%; (b) NaOEt, EtOH, 69%.



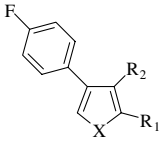
Scheme 6. Reagents and conditions: (a) Br_2, $AlCl_3$, $CHCl_3$, 98%; (b) *p*-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME–H_2O, 97%; (c) $N_2H_4 \cdot H_2O$, diethylene glycol, 27%.

conditions gave **34**. Treatment of **34** with hydrazine afforded 2-ethyl-thiophene **35** (Scheme 6).

To evaluate the potential improvement of anthelmintic activity by replacing the thiophene ring, a variety of analogues of **1** and **19** were synthesized (Tables 3–5). Pyrroles **44** and **45** were synthesized from 4-fluorobenzaldehyde (**40**) according to the procedure described in Scheme 7. Knoevenagel condensation of **40** with acetonitrile provided the α,β-unsaturated nitrile **41**,[13] which was converted into the 2-(trimethylstannyl)pyrrole **42** via cycloaddition with a stannylated derivative of tosylmethyl isocyanide.[14] *N*-Methylation followed by replacement of the trimethyltin group with a bromine, via reaction with *N*-bromosuccinimide[14] provided **43**, which was converted into pyrroles **44** and **45**.[15] Furan **49** was obtained from 4-fluoroacetophenone (**46**) via the preparation of the α-oxo ketene dithioacetal intermediate **48** (Scheme 8).[16] Imidazole **55** was synthesized by treatment of 2-chloro-4-fluoroacetophenone with 2-methylisothiourea, potassium carbonate and sodium iodide (50% yield).

The biphenyl analogue **59** was synthesized in two steps from 2-methylsulfanyl-benzonitrile (**57**) as shown in Scheme 9. Biphenyl analogues **60** and **61** were obtained by Suzuki coupling of *p*-fluorophenylboronic acid with 1-bromo-4-methylsulfanyl-benzene (46% yield) and 1-bromo-3-methylsulfanyl-benzene (50% yield), respectively. Finally, the 5-methyl-thiophene **62** (Fig. 2) was obtained by methylation of **1** with *n*-BuLi and dimethyl sulfate (28% yield).

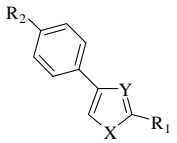
Table 3. In vitro anthelmintic activity of analogues of **1** and **19** with different heterocyclic replacements of the thiophene ring



Compound	X	R_1	R_2	LMA 24 h EC_{50} (μM)	LMA 2 h EC_{50} (μM)
2				15	18
1	S	SMe	CN	28	>200
44	NMe	SMe	CN	175	>200
45	NMe	SEt	CN	NA	NA
19	S	SMe	H	68	>200
49	O	SMe	H	NA	—

NA: Less than 50% of inhibition of the motility at 100 ppm after 24 h of incubation using the LMA.

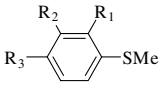
Table 4. In vitro anthelmintic activity of analogues of **5** with different heterocyclic replacements of the thiophene ring and different substituents at the 2- and 4-positions of the five-membered ring



Compound	X	Y	R_1	R_2	LMA 24 h EC_{50} (μM)	LMA 2 h EC_{50} (μM)
2					15	18
1					28	>200
5	S	CH	SEt	H	NA	—
6	S	CH	SEt	F	12	>200
19	S	CH	SMe	F	68	>200
50	S	N	SMe	H	190	82
51	S	N	SEt	H	32	>200
52	S	N	NHMe	H	65	46
53	S	N	NHMe	F	64	98
54	S	N	NHEt	H	102	162
55	NH	N	SMe	F	NA	—
56	NMe	N	SMe	F	NA	—

NA: Less than 50% of inhibition of the motility at 100 ppm after 24 h of incubation using the LMA.

Table 5. In vitro anthelmintic activity of analogues of **1** and **19** with phenyl replacement of the thiophene ring

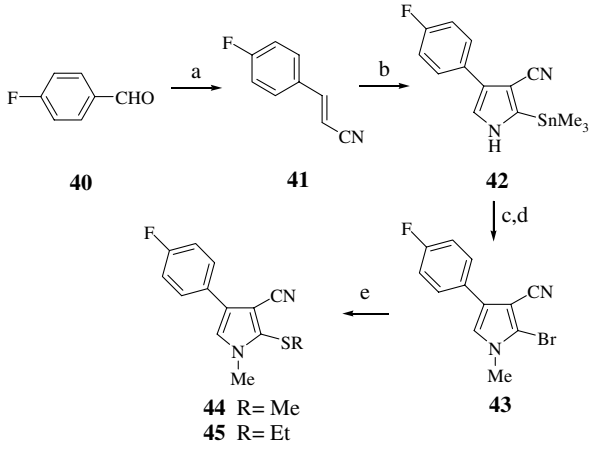


Compound	R_1	R_2	R_3	LMA 24 h EC_{50} (μM)	LMA 2 h EC_{50} (μM)
2				15	18
1				28	>200
19				68	>200
59	CN	H	*p*-F-Ph	NA	—
60	H	H	*p*-F-Ph	NA	—
61	H	*p*-F-Ph	H	NA	—

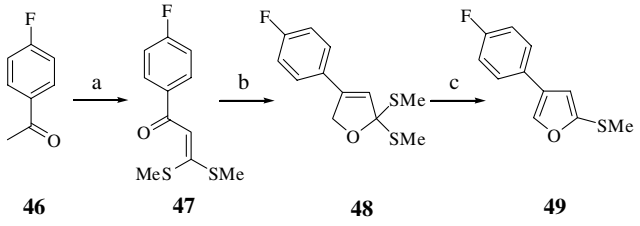
NA: Less than 50% of inhibition of the motility at 100 ppm after 24 h of incubation using the LMA.

Analogues of **1** were initially screened in the LMA at 100 ppm after 24 h of incubation, compounds that showed more than 50% of inhibition of the motility were considered active. All active compounds were tested at different concentrations to determine their EC_{50} after 2 and 24 h of incubation. Although we were interested in compounds with potent in vitro activity after 2 h of incubation, we used EC_{50} values after 24 h of incubation as a tool to guide the SAR of molecules with less potent in vitro anthelmintic activity. A selection of the most potent compounds with EC_{50} <100 μM after 2 or 24 h of incubation were tested in a in vivo gerbil model against *H. contortus* and *T. colubriformis*.

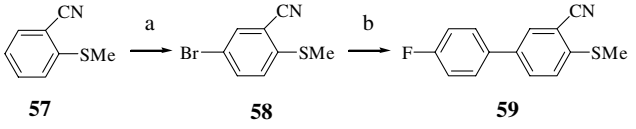
First, analogues of **1** with different substituents at the 2- and 3-positions of the thiophene ring were examined (Table 1). The in vitro anthelmintic activity of analogues with hydrogen (**19**) and bromine (**20**) at the 3-position of the thiophene ring was comparable to the biological activity of the initial lead **1**. However, the



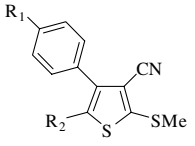
Scheme 7. Reagents and conditions: (a) CH_3CN, KOH, 94%; (b) $(CH_3)_3SnCl$, *n*-BuLi, tosylmethyl isocyanide, 40%; (c) CH_3I, KOH, CH_2Cl_2, 52%; (d) NBS, THF, 62%; (e) RSSR, *n*-BuLi, Et_2O, 59% (**44**), 35% (**45**).



Scheme 8. Reagents and conditions: (a) CS_2, NaO^{t}Bu, CH_3I, benzene–DMF, 66%; (b) *n*-BuLi, $S(CH_3)_3BF_4$, THF; (c) CH_3OH, HCl (2 N), 63% (two steps).



Scheme 9. Reagents and conditions: (a) NBS, HBF_4·OMe, CH_3CN, 55%; (b) *p*-fluorophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O, 93%.



36 R_1 = H, R_2 = H
37 R_1 = CF_3, R_2 = H
38 R_1 = Cl, R_2 = H
39 R_1 = CH_3, R_2 = H
62 R_1 = CH_3, R_2 = CH

Figure 2. Thiophenes **36**–**39**, and **62**.

3-nitro-thiophene **21**[17] was inactive at 100 ppm after 24 h of incubation. These observations indicate that the size and electronic nature of the group at the 3-position of the thiophene may be critical for the anthelmintic activity. The in vitro anthelmintic activity of the 2-ethylsulfanyl-thiophene **23** was slightly better than the lead compound **1**. However, other analogues of **1** with different groups at the 2-position of the thiophene such as hydrogen (**26**), amino (**28**), methylamino (**29**), methanesulfonyl (**30**), and ethoxy (**31**) did not cause significant anthelmintic activity at 100 ppm after 24 h of incubation. In addition, replacement of the methylsulfanyl group at the 2-position of thiophene **19** with acetyl (**34**) or ethyl (**35**) was detrimental to the in vitro activity (Table 1). Presumably, a small alkylsulfanyl group at the 2-position of the thiophene ring is essential for the in vitro anthelmintic activity against *H. contortus*.

In our initial screening effort, we found that analogues of **1** with different substituents at the *para* position of the phenyl ring such as hydrogen (**36**),[3] trifluoromethyl (**37**),[3] chlorine (**38**),[3] and methyl (**39**)[3] (Fig. 2), did not cause significant anthelmintic activity at 100 ppm after 24 h of incubation.

To further evaluate the impact of other groups at the 4-position of the thiophene ring, a variety of analogues of 2-ethylsulfanyl-4-phenyl-thiophene (**5**), with different groups at the *ortho*, *meta*, and *para* positions of the phenyl ring, were tested in the LMA (Table 2).

The best results were obtained with the *p*-F-phenyl (**6**), the *p*-NO_2-phenyl (**7**), the *m*-CN-phenyl (**9**), and the *m*,*p*-diF-phenyl (**16**) substituents at the 4-position of the thiophene ring. However, other *meta* substituents such as fluorine (**8**), methyl (**10**), trifluoromethyl (**11**), and nitro (**12**), did not cause significant anthelmintic activity at 100 ppm after 24 h of incubation using the LMA. Analogues of **5** containing fluorine (**13**), nitrile (**14**), and trifluoromethyl (**15**) at the *ortho* position of the phenyl ring were inactive at 100 ppm after 24 h of incubation. Apparently, certain electron-withdrawing groups at the *para* and *meta* position of the phenyl ring are desirable for in vitro anthelmintic activity. Among them, analogues containing *p*-F-phenyl (**6**), *p*-NO_2-phenyl (**7**), and *m*,*p*-diF-phenyl (**16**) at the 4-position of the thiophene ring provided the best in vitro anthelmintic activity. The replacement of the phenyl ring of **5** with a 2-thienyl group caused a slight increase in anthelmintic activity. Interestingly, the 3-pyridyl analogue **18** had similar in vitro anthelmintic activity as levamisole (**2**) after 2 h of incubation (Table 2).

Next, different replacements of the thiophene ring were investigated as indicated in Tables 3–5. Replacement of the thiophene ring of **1** with a pyrrole ring lead to a decrease in anthelmintic activity (Table 3). Pyrrole **44** showed moderate anthelmintic activity after 24 h of incubation and pyrrole **45** did not show significant anthelmintic activity at 100 ppm after 24 h of incubation. Furan **49** had less anthelmintic activity than its thiophene analogue **19** (Table 3). The better anthelmintic activity observed with analogues containing thiophene versus pyrrole or furan may be related with the larger bonding radius of sulfur leading to more aromatic character of the ring and higher stability.

A number of five-membered rings containing more than one hetero atom were also evaluated (Table 4). The

2-methylsulfanyl-thiazole **50**[18] and the 2-ethylsulfanyl-thiazole **51**[19] showed potent in vitro activity after 24 h, whereas the 2-ethylsulfanyl-thiophene **5** was inactive at 100 ppm after 24 h of incubation (Table 4). Surprisingly, 2-methylamino-thiazoles **52**[20] and **53**[21] and the 2-ethylamino-thiazole **54**[21] showed potent in vitro anthelmintic activity after 24 and 2 h of incubation (Table 4), whereas the 2-methylamino-thiophene **29** was inactive at 100 ppm after 24 h of incubation (Table 1). In the thiazole series the *p*-F-phenyl substituent at the 4-position of the thiazole ring is not essential for the anthelmintic activity as shown by the potent anthelmintic activity of compounds **50**–**52** and **54**. Indeed, 4-phenyl-thiazole **52** and 4-(*p*-F-phenyl)-thiazole **53** had similar in vitro anthelmintic activity, in contrast with their thiophene analogues (compounds **5** and **6**, respectively). Among the thiazoles tested, analogue **52** had comparable in vitro potency to levamisole (**2**) after 2 h of incubation. Replacement of the thiophene ring of **5** with a thiazole ring (compound **51**) lead to an increase in the anthelmintic activity. However, replacement of the thiophene ring of **19** with an imidazole ring (compound **56**[22]), lead to a significant decrease in anthelmintic activity (Table 4). This is surprising considering that pyrrole **44** showed only a slight decrease in activity in comparison to its thiophene analogue **1** (Table 3).

Biphenyl analogues **59**, **60**, and **61** showed less than 50% of inhibition of the motility at 100 ppm after 24 h of incubation (Table 5). These observations suggest that the orientation of the substituents around the core ring and the electronic nature of the core ring are important for the anthelmintic activity. The 5-methyl-thiophene **62** (Fig. 2) did not show significant anthelmintic activity after 24 h of incubation.

Furthermore, a selection of compounds, that exhibited potent in vitro anthelmintic activity against *H. contortus*, was tested in a gerbil in vivo model against *H. contortus* and *T. colubriformis*. Thiophenes **1**, **19**, **20**, **6**, **9**, **16**, and **18** and thiazoles **50** and **52** did not cause a significant reduction of the number of nematodes (*H. contortus* or *T. colubriformis*) at 100 mg/kg, whereas levamisole (**2**) is completely efficacious at 10 mg/kg. Compounds **20**, **6**, **50**, and **52** caused high levels of gerbil mortality at 100 mg/kg. Indeed, compounds **18** and **52**, with potent in vitro anthelmintic activity after only 2 h of incubation, were also inactive in the gerbil in vivo model. This inability to demonstrate a correlation between potent in vitro activity and in vivo efficacy, coupled with the high levels of mortality observed with some analogues, discouraged the evaluation of other potent in vitro anthelmintic compounds in the gerbil assay. The lack of correlation between in vitro and in vivo anthelmintic activity could be related to a number of factors. The insufficient exposure of the compounds to the nematodes in the stomach (*H. contortus*) or intestine (*T. colubriformis*) and the potential different anthelmintic efficacy against L3 stage larvae used in the LMA and adult nematodes (L4 stage) in vivo, can be critical factors for the lack of in vivo anthelmintic efficacy of this new family of compounds. In addition, other biological effects of these molecules may play a role in the lack of in vivo activity, as for example, reversible/irreversible character of the inhibition of the motility and increase/decrease capacity of feeding.

In summary, we have described the synthesis and anthelmintic activity against *H. contortus* of analogues of a novel thiophene lead structure (**1**). Some compounds showed potent in vitro anthelmintic activity, but their in vitro efficacy did not translate into significant in vivo activity. It is worthwhile to explore these compounds as novel anthelmintic agents and further study their pharmacological actions to improve their in vivo activity.

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4. *In vitro anthelmintic efficacy assay*: Third-stage (L3) *H. contortus* larvae were obtained from the feces of infected sheep, purified and maintained, at 4 °C, in phosphate-buffered saline (PBS) until use. L3 larvae were pipetted into wells (30–40 larvae/well) of 96-well dishes containing the compounds and incubated at 27 °C for 2 or 24 h. Following this, the larvae were transferred to a 96-well insert. The bottom of each well in the insert is nylon mesh with an average pore size of 40 μm, overlaid with 75 μL of 2% low melt agar. Following an additional 4 h incubation, the insert was removed and the larvae capable of migrating through the mesh-agar barrier were counted. EC_{50} values were determined by 2-fold serial dilutions from 200 to 0.2 μM.
5. *In vivo anthelmintic efficacy assay*: In vivo efficacy was evaluated in gerbils infected with both *H. contortus* and *T. colubriformis* larvae. The animals were immunocompromised by addition of 0.02% hydrocortisone to the feed 7 days pre-infection. Gerbils were inoculated with a mixture of 1500 exsheathed, third-stage *H. contortus* larvae and 1500 ensheathed, third-stage *T. colubriformis* larvae. 7 days post-infection, compounds were administered orally at 100 and 50 mg/kg. All animals were euthanized 3 days following compound administration and the number of nematodes in the stomach and large intestine determined.
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7. Synthesis of thiophenes **5**–**18**: *n*-BuLi (2.5 M in hexane, 2.7 mL, 6.70 mmol) was added dropwise to a stirred solution of 2,4-dibromothiophene (1.49 g, 6.20 mmol) in dry ether (15 mL) at −70 °C. After 0.5 h, diethyl disulfide (0.82 g, 6.70 mmol) in dry ether (2 mL) was added. The

reaction mixture was held at −70 °C for 2 h, allowed to warm to −35 °C over 2 h, and added to a mixture of NH_4Cl in ice. The organic phase was separated, the aqueous phase was re-extracted with Et_2O, and the combined ether solutions were washed with saturated solution of NaCl, dried over $MgSO_4$, filtered, and concentrated Column chromatography (silica, hexane/EtOAc = 95/5) gave **4** as a colorless liquid (1.15 g, 5.14 mmol, 83%): ^1H NMR ($CDCl_3$, 400 MHz): δ 1.32 (t, 3H, $J = 7.4$ Hz), 2.86 (q, 2H, $J = 7.4$ Hz), 7.05 (d, 1H, $J = 1.2$ Hz), 7.26 (d, 1H, $J = 1.2$ Hz). To a stirred solution of **4** (0.20 g, 0.89 mmol) in ethylene glycol dimethyl ether (4 mL) and $Pd(PPh_3)_4$ (0.05 g, 0.05 mmol) were added Na_2CO_3 (0.25 g, 2.33 mmol) in water (2 mL) and *p*-fluorophenylboronic acid (0.16 g, 1.16 mmol). After stirring at 80 °C for 24 h, $Pd(PPh_3)_4$ (0.05 g, 0.05 mmol) was added to the reaction mixture followed by Na_2CO_3 (0.12 g, 1.16 mmol) in water (1 mL) and *p*-fluorophenylboronic acid (0.08 g, 0.58 mmol). After stirring at 80 °C for 8 h, the reaction mixture was cooled to room temperature, diluted with water, and extracted with EtOAc. The organic phase was washed with saturated solution of NaCl, dried over $MgSO_4$, filtered, and concentrated. Chromatography (silica, hexane/EtOAc = 97/3) provided **6** as an oil (0.12 g, 0.49 mmol, 55%): ^1H NMR ($CDCl_3$, 400 MHz): δ 1.32 (t, 3H, $J = 7.4$ Hz), 2.86 (q, 2H, $J = 7.4$ Hz), 7.06–7.09 (m, 2H), 7.35 (d, 1H, $J = 1.6$ Hz), 7.37 (d, 1H, $J = 1.6$ Hz), 7.48–7.52 (m, 2H); HRMS calculated for $C_{12}H_{11}FS_2$ $[M]^+$ 238.0286, found 238.0290; HPLC [column Zorbax SB-C-18 (4.6 × 75 mm, 3.5 μm), $T = 22$ °C, linear gradient: 10–90%B (25 min), 90%B (5 min) (A = 0.05% TFA in water, B = CH_3CN), 1.5 mL/min] $t_R = 23.5$ min.

Using a similar procedure from **4** (50 mg, 0.22 mmol) compound **18** was obtained as an oil (24 mg, 0.11 mmol, 50%): ^1H NMR ($CDCl_3$, 400 MHz): δ 1.32 (t, 3H, $J = 7.4$ Hz), 2.88 (q, 2H, $J = 7.4$ Hz), 7.32 (ddd, 1H, $J_1 = 0.8$, $J_2 = 5.2$, $J_3 = 8$ Hz), 7.39 (d, 1H, $J = 1.6$ Hz), 7.51 (d, 1H, $J = 1.6$ Hz), 7.82 (ddd, 1H, $J_1 = 1.6$, $J_2 = 2.4$, $J_3 = 8$ Hz), 8.53 (dd, 1H, $J_1 = 1.6$, $J_2 = 5.2$ Hz), 8.83 (dd, 1H, $J_1 = 0.8$, $J_2 = 2.4$ Hz); HRMS calculated for $C_{11}H_{11}NS_2$ $[M]^+$ 221.0333, found 221.0332; HPLC [HPLC conditions as for **6**] $t_R = 10.5$ min.

Compounds **5** and **7**–**17** were prepared by reacting **4** with appropriate arylboronic acids by Suzuki coupling as similarly described for compound **6**. Compounds **7**–**8** and **10**–**17** were synthesized in parallel using a QUEST instrument and yields were not optimized.

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9. Synthesis of thiophene **23**: NBS (0.19 g, 1.05 mmol) was added in portions to a solution of **6** (0.10 g, 0.42 mmol) in THF (5 mL) at −20 °C. The mixture was allowed to warm to room temperature. After 24 h the reaction mixture was concentrated, diluted with water and extracted with EtOAc. The combined extracts were washed with saturated solution of NaCl, dried over $MgSO_4$, filtered, and concentrated. The oil obtained was dissolved in AcOH–THF–H_2O (2.5:1:7) (0.85 mL), Zn dust (35 mg, 0.54 mmol) was added and the reaction mixture was heated at reflux for 8 h. The mixture was cooled to room temperature, diluted with water, and extracted with EtOAc. The organic phase was washed with saturated solution of NaCl, dried over $MgSO_4$, filtered, and concentrated. Chromatography (silica, hexane) provided **22** (42 mg, 0.13 mmol, 31%) as an oil. CuCN (17 mg, 0.19 mmol) was added to a solution of **22** (40 mg, 0.13 mmol) in NMP (1 mL). After 3 h of stirring at reflux, CuCN (17 mg, 0.19 mmol) was added. The reaction mixture was stirred at reflux for 5 h, allowed to cool to room temperature. A solution of 28% NH_3 in water (0.5 mL) diluted in water (0.5 mL) was added. After 30 min the reaction mixture was filtered, and the filtrate was extracted with DCM. The organic phase was washed with saturated solution of NaCl, dried over $MgSO_4$, filtered, and concentrated. Chromatography (silica, hexane/EtOAc = 95/5) provided **23** as an oil (12 mg, 0.05 mmol, 36%): ^1H NMR ($CDCl_3$, 400 MHz): δ 1.43 (t, 3H, $J = 7.4$ Hz), 3.10 (q, 2H, $J = 7.4$ Hz), 7.16–7.20 (m, 2H), 7.31 (s, 1H), 7.58–7.62 (m, 2H); GC–MS 263 $[M]^+$.
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15. Synthesis of pyrroles **44** and **45**: *n*-BuLi (2.5 M in hexane, 2.83 mL, 7.09 mmol) was added dropwise to a solution of tosylmethyl isocyanide (0.66 g, 3.38 mmol) in THF (50 mL) at −78 °C. After 5 min Me_3SnCl (1.41 g, 7.09 mmol) in THF (2 mL) was added dropwise. After another 5 min, a solution of **41** (0.49 g, 3.38 mmol) in THF (2 mL) was added dropwise. The temperature of the reaction mixture was allowed to rise to room temperature in 30 min, and stirring was continued for 4 h. Water was added, and the mixture was extracted with EtOAc. The combined extracts were washed with water and with saturated solution of NaCl, dried over $MgSO_4$, filtered, and concentrated. Chromatography (alumina, CH_2Cl_2) gave **42** (0.47 g, 1.35 mmol, 40%) as an oil: ^1H NMR ($CDCl_3$, 400 MHz): δ 0.49 (s, 9H), 7.06–7.11 (m, 3H), 7.56–7.59 (m, 2H). A solution of KOH (50% in water, 3.35 mL) was added to a solution of **42** (0.39 g, 1.23 mmol), benzyltriethylammonium chloride (0.04 g, 0.17 mmol), and MeI (0.56 mL, 9.01 mmol) in CH_2Cl_2 (11 mL). The suspension was stirred vigorously for 1 h. Water was added, and the organic layer was separated, dried over $MgSO_4$, filtered, and concentrated. Chromatography (alumina, hexane/EtOAc = 90/10) gave 4-(4-fluorophenyl)-1-methyl-2-trimethylstannyl-1*H*-pyrrole-3-carbonitrile (0.22 g, 0.62 mmol, 52%) as an oil: ^1H NMR ($CDCl_3$, 400 MHz): δ 0.52 (s, 9H), 3.72 (s, 3H), 6.89 (s, 1H), 7.05–7.09 (m, 2H), 7.52–7.55 (m, 2H). A solution of 4-(4-fluorophenyl)-1-methyl-2-trimethylstannyl-1*H*-pyrrole-3-carbonitrile (0.22 g, 0.62 mmol) in THF (4 mL) was cooled to −78 °C. NBS (0.12 g, 0.68 mmol) was added in small portions and the reaction mixture was stirred for 30 min at −78 °C. The mixture was allowed to warm to 0 °C and after 20 h water was added and the mixture was extracted with Et_2O. The combined organic layers were washed with saturated solution of NaCl, dried over $MgSO_4$, filtered, and concentrated. Chromatography (alumina, hexane/EtOAc = 85/15) gave **43** (0.11 g, 0.38 mmol, 62%) as an oil: ^1H NMR ($CDCl_3$, 400 MHz): δ 3.69 (s, 3H), 6.86 (s, 1H), 7.06–7.11 (m, 2H), 7.51–7.54 (m, 2H). *n*-BuLi (2.5 M in hexane, 72 μL, 0.18 mmol) was added dropwise to a stirred solution of **43** (48 mg, 0.17 mmol) in dry ether (1 mL) at −78 °C. After 0.5 h, dimethyl disulfide (57 μL, 0.19 mmol) was added. The reaction mixture was held at −70 °C for 0.5 h, allowed to warm to room temperature in 4 h, and added to a mixture of NH_4Cl in ice. The organic phase was separated, the aqueous phase was re-extracted with Et_2O, and the combined ether solutions were washed with saturated NaCl solution, dried over $MgSO_4$, filtered, and concentrated. Column chromatography (silica, hexane/EtOAc = 90/10) gave **44** as an oil (25 mg, 0.10 mmol,

59%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.44 (s, 3H), 3.77 (s, 3H), 6.89 (s, 1H), 7.06–7.11 (m, 2H), 7.53–7.57 (m, 2H); HRMS calculated for $C_{13}H_{11}N_2FS$ $[M]^+$ 246.0627, found 246.0630; HPLC [column YMC ODS-AQ C-18 (4.6×150 mm, 5 μm), $T = 22$ °C, linear gradient: 10–100%B (20 min), 100%B (10 min) (A = 0.01% CF_3COOH in water, B = CH_3CN), 1 mL/min] $t_R = 19.4$ min.

Using a similar procedure from **43** (49 mg, 0.17 mmol) compound **45** was obtained as an oil (16 mg, 0.06 mmol, 35%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.28 (t, 3H, J = 7.2 Hz), 2.84 (q, 2H, J = 7.2 Hz), 3.76 (s, 3H), 6.92 (s, 1H), 7.06–7.11 (m, 2H), 7.55–7.58 (m, 2H); HRMS calculated for $C_{14}H_{13}N_2FS$ $[M]^+$ 260.0783, found 246.0773; HPLC [HPLC conditions as for **44**] $t_R = 20.2$ min.

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