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Discovery of amino-acetonitrile derivatives, a new class of synthetic anthelmintic compounds

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Abstract—A new series of amino-acetonitrile derivatives (AAD) have been discovered that exhibit high anthelmintic activity against parasitic nematode species such as *Haemonchus contortus* and *Trichostrongylus colubriformis*. Significantly, these compounds also demonstrate activity against nematode strains resistant to the currently available broad-spectrum anthelmintics. The discovery, synthesis, structure–activity relationship and biological results are presented.

Parasitic nematodes, apart from being one of the most frequent sources of human infections particularly in tropical countries are also a major cause of disease and productivity loss in farmed livestock. While only three classes of broad-spectrum anthelmintic drugs are widely used at present (benzimidazoles, imidazothiazoles and macrocyclic lactones), resistance against the pertinent compounds has become a serious concern in veterinary medicine.[1] Thus, the discovery of novel molecules able to control multi-resistant nematodes is of prime importance.[2]

In the course of our search for new anthelmintics, the amino-acetonitrile derivative **1**[3] (Fig. 1) was identified as a lead compound in a larval development assay[4] (LDA) against a benzimidazole-resistant isolate of *Haemonchus contortus* (*H.c.*). Certain *N*-acyl amino-acetonitriles had been associated with diverse biological properties (e.g. insecticides,[5] fungicides, [6] antibacterials[7] as well as cysteine cathepsin inhibitors[8]), but have never been described as nematocides.

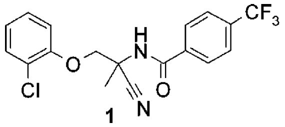

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Figure 1.

Anthelmintic efficacy was also confirmed in a rodent model.[9] Compound **1** showed a significant reduction in the number of *H.c.* but no reduction of *Trichostrongylus colubriformis* (*T.c.*) following oral administration as a racemate at 10 mg/kg bodyweight. These encouraging biological results prompted a chemical optimization program with the aim of increasing the potency and spectrum of activity.

Analogues were readily prepared by the alkylation of a phenol with chloroacetone, followed by Strecker reaction on the resulting ketone and a final acylation step of the amino group using an acid chloride (Scheme 1).[10,11] Although it was anticipated that the enantiomers would exhibit different levels of efficacy, synthesis and initial biological evaluations were mainly performed with the racemates.

Alternatively, in case starting phenols were not easily accessible and the R^1 substituents were electron-with-

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Keywords: Amino-acetonitrile derivatives; AAD; Anthelmintic; Parasiticide; Nematocide; *Haemonchus contortus*.

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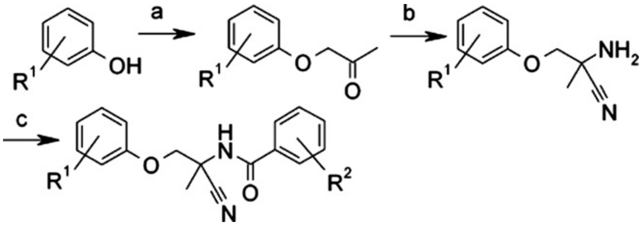
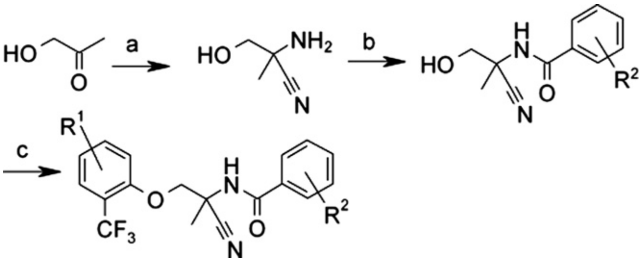


Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, chloroacetone, acetone, Rfl.; (b) NH_4Cl, NaCN, NH_3/H_2O; (c) DIPEA, 4-DMAP, $R^2ArCOCl$, CH_2Cl_2.



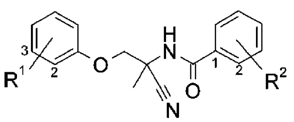
Scheme 2. Reagents and conditions: (a) NH_4Cl, NaCN, $NH_3/MeOH$; (b) 1 M $NaHCO_3$, EtOAc, $R^2ArCOCl$; (c) *t*-BuOK, 2-CF_3-R^1ArF, THF.

drawing, a second route via aromatic nucleophilic substitution (S_NAr) was used (Scheme 2). Strecker reaction of hydroxyacetone followed by chemo selective N-benzoylation with substituted benzoyl chlorides gave the corresponding hydroxy-benzamide derivatives. Finally, aromatic substitution of activated fluorobenzenes afforded the targeted analogues.[12,13]

In order to understand the structural requirements, a first series of compounds bearing different R^1 substituents on the phenoxy moiety was prepared (**2**–**24**) and evaluated in the LDA (Table 1). At the 2-position electron-withdrawing groups (**1**–**4**) afforded better activity than electron donating groups. An increased potency was observed in the case of CF_3 compared to Cl. No substituent at this position led to decreased activity in any cases (**6**, **9** and **10**). For this reason, the 2-Cl group was kept at first for optimization of the other positions on this ring. Position 6 was very sensitive to substitution and steric hindrance, yielding to a loss of activity with any group except for H (**15** and **16**). Slight decrease of intrinsic activity and no improvement of efficacy in gerbils were observed with groups at position 3 (**8**) (Table 2). Remarkably, the small electron-withdrawing groups F and CN at positions 4 and/or 5 (**12**, **14** and **17**–**24**) led to a moderate increase of in vitro efficacy along with a strong increase of in vivo efficacy.

To further explore the SAR, another series of compounds (**25**–**36**) with modifications of the R^2 substituent on the benzamide moiety was synthesized. Substitutions at *ortho* and *meta* positions led to inactive compounds (**25**, **27**) or to a decreased activity in case the *para* CF_3-substituent was present (**26**). At the 4-position the substituents could be ranked as follows: $CF_3 \cong OCF_3 \cong SCF_3 > Cl \gg F > H = Me$.

Table 1. Activity of compounds **1**–**36** against *H. contortus* and *T. colubriformis* in LDA



Compound	R^1	R^2	*H.c.* EC_{100}[a,b] (ppm)	*T.c.* EC_{100}[a,b,c] (ppm)
Thiabendazole	—	—	10	1
Levamisole	—	—	0.32	10
Ivermectin	—	—	0.001	0.01
1	2-Cl	4-CF_3	0.032	0.032
2	2-Cl	4-OCF_3	0.032	0.032
3	2-CF_3	4-OCF_3	0.01	0.01
4	2-Br	4-OCF_3	0.032	0.032
5	2-F	4-CF_3	1	1
6	H	4-CF_3	>10	>10
7	2-Et	4-OCF_3	0.32	0.32
8	2,3-diCl	4-CF_3	0.1	0.1
9	3-F	4-OCF_3	0.32	1
10	4-CF_3	4-CF_3	>10	>10
11	2,4-diCl	4-OCF_3	0.1	0.1
12	2-Cl, 4-F	4-OCF_3	0.032	0.032
13	2,5-diCl	4-OCF_3	0.01	0.032
14	2-Cl, 5-F	4-OCF_3	0.01	0.032
15	2,6-diCl	4-CF_3	>10	>10
16	2,4,6-triF	4-OCF_3	>10	>10
17	2-Br, 5-F	4-OCF_3	0.01	0.1
18	2-Br, 4,5-diF	4-OCF_3	0.01	0.032
19	2-CF_3, 4,5-diF	4-OCF_3	0.01	0.032
20	2-CF_3, 4,5-diF	4-CF_3	0.032	0.032
21	2-CF_3, 4-CN	4-CF_3	0.1	0.1
22	2-CF_3, 4-CN	4-OCF_3	0.032	0.1
23	2-CF_3, 5-CN	4-CF_3	0.032	0.1
24	2-CF_3, 5-CN	4-OCF_3	0.01	0.032
25	2-Cl	2-CF_3	>10	>10
26	2-Cl	2-F, 4-CF_3	0.32	0.32
27	2-Cl	3-OCF_3	>10	10
28	2-Cl	4-Me	>10	>10
29	2-Cl	4-F	3.2	1
30	2-CF_3	H	10	>10
31	2-Cl	4-Cl	0.32	0.32
32	2-CF_3	4-Ph	>10	>10
33	2-CF_3	4-SCF_3	0.032	0.032
34	2-CF_3, 4,5-diF	4-SCF_3	0.032	0.032
35	2-CF_3, 4-CN	4-SCF_3	0.032	0.032
36	2-CF_3, 5-CN	4-SCF_3	0.01	0.032

[a] Values are means of three experiments.
[b] Benzimidazole-resistant isolate.
[c] Levamisole-resistant isolate.

Compounds active following oral application in gerbils at 1 mg/kg bodyweight (**19**–**20**, **22**–**24** and **34**–**36**) were also evaluated in the gerbil model after subcutaneous injection to investigate their systemic efficacy (Table 3).

Systemic activity was indeed observed, with slight differences between these analogues. These may be due to small changes in the compounds' polarity and thus, in their pharmacokinetic profiles in rodents.

Racemic **19** and **36** were separated using chiral HPLC. The most active enantiomers showed at least a 300-fold

Table 2. Activity of selected analogues of compound **1** against *H. contortus* and *T. colubriformis* in gerbils after oral treatment

Compound	Dose (mg/kg)	*H.c.* (% worm count reduction)	*T.c.* (% worm count reduction)
Ivermectin	0.1	98	97
1	10	88	19
3	10	98	97
4	10	97	99
8	10	84	67
11	10	94	100
12	3.2	98	77
13	3.2	68	89
14	3.2	92	79
17	3.2	90	99
18	3.2	100	97
19	1	99	73
20	1	89	91
21	1	97	91
22	1	95	98
23	1	97	87
24	1	95	85
33	10	77	99
34	1	84	98
35	1	90	98
36	1	100	100

Table 3. Activity of selected analogues of compound **1** against *H. contortus* and *T. colubriformis* in gerbils after subcutaneous treatment

Compound	Dose (mg/kg)	*H.c.* (% worm count reduction)	*T.c.* (% worm count reduction)
Ivermectin	0.32	99	85
19	1	99	75
20	1	94	90
22	1	91	97
23	1	90	72
24	1	97	75
34	1	73	90
35	1	92	86
36	1	99	84

higher potency in the LDA (Table 4). Trace amount of the (−)-enantiomers may be the cause of the residual efficacy of the (+)-enantiomers. These results tend to demonstrate enantiospecificity of these compounds against nematodes.

The absolute configuration (*S*) of the most active enantiomer of **36** was established by single crystal X-ray structure determination (Fig. 2).[14a]

Table 4. Activity of separated enantiomers of **19** and **36** against *H. contortus* and *T. colubriformis* in LDA

Compound[a]	*H.c.* EC_{100}[b,c] (ppm)	*T.c.* EC_{100}[b,c,d] (ppm)
(+)-**19**	1	3.2
(−)-**19**	0.0032	0.01
(***R***)-(+)-**36**	10	>10
(***S***)-(−)-**36**	0.0032	0.01

[a] ee > 99.0% as determined by analytical chiral HPLC.

[b] Values are means of three experiments.

[c] Benzimidazole-resistant isolate.

[d] Levamisole-resistant isolate.

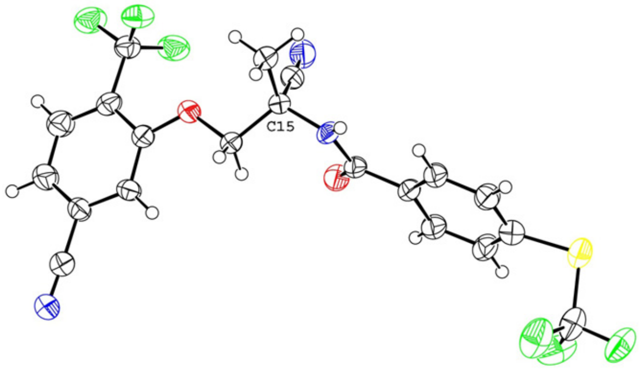
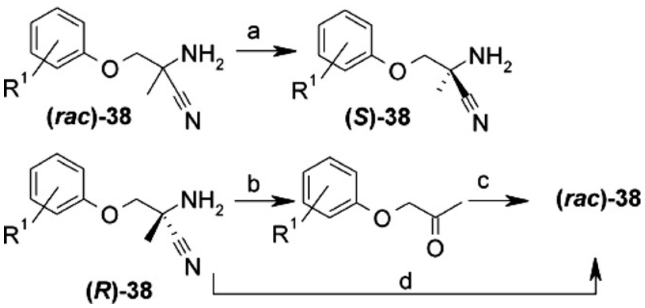


Figure 2. Structure of compound **36**[15] in the crystal.[14b] For the (*S*)-enantiomer shown the Flack *x* parameter refined to −0.02(2).[14c]



Scheme 3. Reagents and conditions: (a) resolution with enantiopure chiral acid; (b) $CuSO_4$, EtOH, Rfl.; (c) NH_4Cl, NaCN, NH_3/MeOH; (d) standing in MeOH.

Several routes leading to the desired enantiomers were analyzed. Although efficient enantioselective Strecker reactions have been reported,[16] the use of often expensive metal catalysts was not considered practical in view of future scale-up and large scale production. Thus, we decided to investigate resolution options. Intermediate compounds **38** were obvious candidates since diastereomeric salts of amino nitriles with chiral acids had been described[17] and used industrially (Scheme 3). Furthermore, the unwanted enantiomers could easily be racemized either spontaneously (standing in MeOH) or via a retro-Strecker using $CuSO_4$/Strecker sequence. In the case of R^1 = 2-CF_3, 5-CN the amine (***S***)-**38** was obtained in 95–98% ee and 40% yield using (+)-di-*O*,*O*′-*p*-toluyl-ᴅ-tartaric acid as the chiral acid. This approach looks promising for large scale production.

In this letter, we have described the discovery of a new series of amino-acetonitrile derivatives (AAD) with significant nematocidal activity. Despite intensive efforts in medicinal chemistry over the past 25 years,[18] merely two new classes of broad-spectrum anthelmintics have emerged, the cyclodepsipeptides[19] and the paraherquamides,[20] both derived from fermentation. However, neither class has resulted in a marketed product for livestock as yet. Several of the compounds disclosed above are currently under evaluation as drug development candidates for use in ruminants. Since the amino-acetonitrile derivatives also possess a new mode of action,[21] they could offer farmers a sought-after solution to overcome current anthelmintic resistance issues. If the favorable tolerability of this class of compounds observed in ruminants can be confirmed for humans, human medical practice may also benefit from this new alternative to current treatments.

Acknowledgments

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9. In vivo anthelmintic efficacy assay in *Meriones unguiculatus*: gerbils were artificially infected with larvae of *H.c.* and *T.c.* 6, respectively, 5 days prior to treatment. Experimental animals were dosed orally or subcutaneously, and control animals received a placebo formulation. Three days post-treatment, gerbils were sacrificed and dissected to recover *H.c.* from the stomach and *T.c.* from the upper part of midgut. Iodine fixed worms were counted after destaining. Efficacy is expressed in percent reduction of worm numbers compared to the geometric mean number of worms collected from the control group, using Abbot's formula.
10. Synthesis of amino-acetonitriles **1**–**20** and **25**–**33**, typical procedures[5]: data for compound **19**: mp = 102–103 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.80 (d, $J = 8.8$ Hz, 2H), 7.47–7.41 (m, 1H), 7.29–7.25 (m, 2H), 6.90 (dd, $J = 11.0$, 6.2 Hz, 1H), 6.54 (br s, 1H), 4.59 (d, $J = 9.0$ Hz, 1H), 4.37 (d, $J = 9.0$ Hz, 1H), 1.96 (s, 3H). HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{19}H_{12}F_8N_2O_3$, 469.0793; found, 469.0793.
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13. Synthesis of amino-acetonitriles **21**–**24** and **34**–**36**, typical procedures[12]: data for compound **36**: mp = 69–73 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (m, 2H), 7.76–7.72 (m, 3H), 7.41 (d, $J = 8.1$ Hz, 1H), 7.30 (s, 1H), 6.50 (br s, 1H), 4.71 (d, $J = 8.9$ Hz, 1H), 4.50 (d, $J = 8.9$ Hz, 1H), 2.00 (s, 3H). HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{20}H_{13}F_6N_3O_2S$, 474.0754; found, 474.0754.
14. (a) Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 676168. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk]; (b) Spek, A. L. *J. Appl. Cryst.* **2003**, *36*, 7; (c) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876.
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