Synthesis and anthelmintic properties of arylquinolines with activity against drug resistant nematodes

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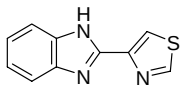
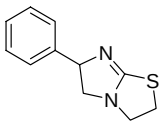
Abstract—2,4-Disubstituted quinolines with additional substituents in positions 5-8 have been found to have anthelmintic properties. A number of 2,4-dimethoxy-6- or 8-arylquinolines have potent activity against the sheep nematode *Haemonchus contortus*, with LD_{99} values of the same order of magnitude as levamisole. These arylquinolines maintain their activity against levamisole, ivermectin and thiabendazole resistant strains of *H contortus*.

Parasitic nematode infections are a continuing threat in both human and animal medicine. The most commonly used classes of drugs to treat such infections are the benzimidazoles such as thiabendazole **1**, imidazothiazoles such as levamisole **2**, and avermectins (obtained from the fermentation products of *Streptomyces avermitilis*). There is evidence of emerging resistance to some drugs in the developing world,[1] and in some areas with high agricultural dependence multiple resistance to all of the major drug classes is appearing in livestock.[2] Alternatives to these classes of drug are now being sought.



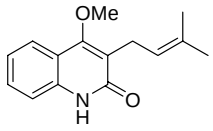
thiabendazole **1**



levamisole **2**

We recently published a concise synthesis of the prenylquinoline alkaloid atanine, **3**,[3] shown to be active against larval stages of the trematode parasite *Schistosoma mansoni*, the cause of schistosomiasis (bilharzia), a major health issue in developing countries.[4]

As part of our program of synthesis and testing of novel atanine analogues, we synthesized a number of intermediate trisubstituted quinolines with substituents in ring positions 5-8. Tests against *S. mansoni* larval stages and the model nematode *C. elegans* revealed that some of these intermediates exhibited anti-nematode activity, differing from the pattern of activity against schistosomes.[5]



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From these early biological results, we became interested in aryl-substituted quinolines, and so we set out to investigate the synthesis of these novel 2,4-dimethoxy aryl-substituted quinolines by exploring the Suzuki coupling of 5-, 6-, 7- or 8-bromoquinoline intermediates.

Substituted-2,4-dimethoxyquinolines were synthesized by condensation/cyclization of the appropriate substituted aniline **4** with malonic acid and phosphoryl chloride to give the 2,4-dichloroquinoline **5**, followed

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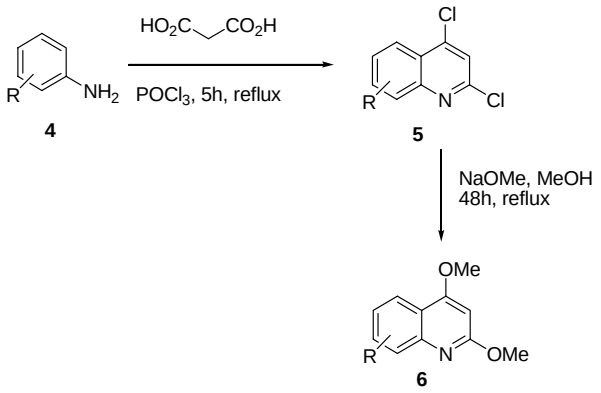
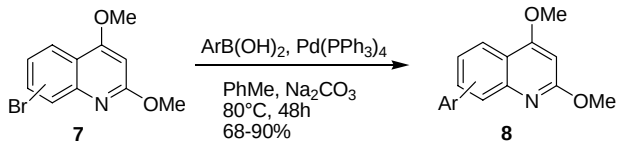
by displacement by methoxide ion to give the required quinoline **6** (Scheme 1).[6]



Scheme 1. Synthesis of substituted-2,4-dimethoxy-quinolines

For our proposed synthesis of arylquinolines, the bromoquinoline precursors were synthesized. Cyclization of 3-bromoaniline gave a mixture of the 5- and 7-bromoquinolines, which were separable by column chromatography.

The bromo-2,4-dimethoxy-quinolines **7** were then coupled to the required aryl groups under Suzuki conditions to yield the arylquinolines **8** (scheme 2). These proceeded in good yield, although it was observed that 8-bromoquinolines were much more sensitive, requiring stricter oxygen-free reaction conditions to give consistent yields.[8]



Scheme 2. Synthesis of arylquinolines via Suzuki coupling

A number of 2,4-disubstituted quinolines (*ca* 80) were tested against the agriculturally important parasitic nematode *Haemonchus contortus*, using the commercial NemaTox larval development screen used for determining drug susceptibility.[9] It was observed that greater than 40% of these compounds exhibited nematocidal activity (LD_{99} <100 μg/mL), with those exhibiting LD_{99} better than 25 μg/mL shown in Table 1. In particular, it appeared that 6- or 8-substituted 2,4-dimethoxyquinolines showed the greatest activity against *H. contortus*. Five compounds, including the arylquinolines **13-16**, had LD_{99} of 12.5 μg/mL or lower in this first screen, showing potential for useful anti-nematode activity. The LD_{99} of compounds **15** and **16**, 3.1 μg/mL, was of the same order as the commercial nematocides levamisole and closantel. These five most active compounds were resynthesized in gram quantities for further investigation.

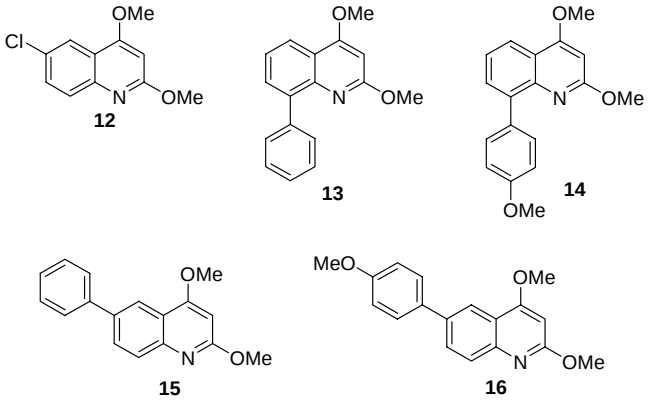


Table 1: LD_{99} values for compounds **9-16** (substituted at positions 2-8) against *H. contortus*

Compound	R2	R3	R4	R5	R6	R7	R8	LD_{99}, μg/mL
9[a]	OMe	Ph	OMe	H	H	H	H	25
10	OMe	H	OMe	Me	H	Me	H	25
11[b]	Cl	H	OMe	H	H	H	H	25
12	OMe	H	OMe	H	Cl	H	H	12.5
13	OMe	H	OMe	H	H	H	Ph	12.5
14	OMe	H	OMe	H	H	H	4-MeO-C_6H_4	12.5
15	OMe	H	OMe	H	Ph	H	H	3.1
16	OMe	H	OMe	H	4-MeO-C_6H_4	H	H	3.1

[a] Synthesized from 2,4-dimethoxyquinoline by bromination at position 3 and subsequent Suzuki coupling.[5, 10]
[b] Product of incomplete substitution of 2,4-dichloroquinoline by methoxide

Table 2: LD_{99} values of compounds **12-16** against susceptible and drug-resistant nematodes

	LD_{99}, µg/mL					
Compound	***H contortus* susceptible**	***H contortus* VSRG, benzimidazole resistant**	***H contortus* Lawes, levamisole and benzimidazole resistant**	***H contortus* CAVR, ivermectin resistant**	***T colubriformis***	***O circumcincta***
12	13	13	8.8	6.3	25	13
13	6.3	4.4	6.3	3.1	50	6.3
14	6.3	6.3	3.1	3.1	6.3	3.1
15	3.1	3.1	3.1	3.1	6.3	3.1
16	3.1	2.2	1.6	1.6	3.1	3.1
1	0.16	5	5	0.16	nt	nt
2	1.6	1.6	>100	0.78	nt	nt

nt: not tested

Compounds **12-16** were tested against susceptible strains of *H. contortus*, strains resistant to benzothiazoles (VSRG strain), ivermectin (CAVR strain) or both benzothiazoles and levamisole (Lawes strain), plus susceptible strains of *T colubriformis* and *O. circumcincta*. Results are shown in Table 2, with activities of thiabendazole **1** and levamisole **2** for comparison.

Of these compounds, **15** and **16** exhibited the greatest activity against susceptible strains of *H. contortus* (3.1µg/mL), comparable in potency to levamisole. **15** and **16** were also potent against the various drug-resistant strains of *H contortus*, with even improved activity for **16**; LD_{99} 1.6 µg/mL, against both the multiple-resistant Lawes strain and the ivermectin resistant CAVR strain. There is also evidence of activity against the important parasitic nematodes *Trichostrongylus colubriformis* and *Ostertagia circumcincta*.

In summary, we have prepared novel arylquinolines in good yields via Suzuki coupling of substituted bromoquinolines. We have demonstrated that a number of these quinolines, in particular the 6-arylquinolines **15**, **16**, show promising potency against susceptible and drug-resistant strains of an important nematode target, and represent a new class of anthelmintic compounds. There is obvious potential for lead optimization and further development to offer a new line of defence against drug-resistant parasitic nematode infections.

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References and notes

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8. Synthesis of arylquinolines: typical procedure: 8-Bromo-2,4-dimethoxyquinoline (0.4 g, 1.5 mmol) was dissolved in toluene (10 mL) under argon. Tetrakis-(triphenylphosphine)palladium (0) (52 mg, 3 mol%) and aqueous sodium carbonate (2 mL of a 2M solution) were added, and the mixture stirred for 5 minutes. Benzeneboronic acid (0.20 g, 1.7 mmol) in ethanol (1 mL) was added, and the mixture was then heated under reflux for 48 hours. After cooling, the mixture was poured into a separating funnel, and the reaction flask washed with water (20 mL) and ether (20 mL); the washings being added to the separating funnel. The aqueous layer was extracted with ether (3 × 20 mL), and the combined organic layers were dried over magnesium sulfate before removal of the solvent under reduced pressure. The crude product was purified by column chromatography (9:1 hexane:EtOAc) to yield 90% of the title compound as white plates, mpt. (CH_2Cl_2/MeOH) 110-112 °C; found M^+: 265.1105, $C_{17}H_{15}NO_2$ requires 265.1103; ^{1}H NMR ($CDCl_3$): δ 8.16 (1H, dd, *J* 8.2, 1.5 Hz, H5), 7.90 (2H, dd, *J* 8.3, 1.5 Hz, H2', H6'), 7.77 (1H, dd, *J* 7.2, 1.5 Hz, H7), 7.54 (2H, m, H6 and H4'), 7.47 (2H, dd, *J* 8.3, 7.2 Hz, H3', H5'), 6.29 (1H, s, H3), 4.01

(3H, s, OMe), 3.99 (3H, s, OMe); ; ^{13}C NMR ($CDCl_3$): δ 164.5, 163.4 (C2, C4), 144.7, 140.4, 138.5 (C1', C8, C8a), 131.3, 131.1, 127.9, 127.2, 123.5, 121.8 (C5, C6, C7, C2'-6'), 120.1 (C4a), 90.7 (C3), 56.2 (OMe), 53.8 (OMe); anal. found C 76.91, H 5.65, N 5.19%, calculated for $C_{17}H_{15}NO_2$ C 76.96, H 5.70, N 5.28%;

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