

Short Report: Antischistosomal versus Antiandrogenic Properties of Aryl Hydantoin Ro 13-3978

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Abstract. In the early 1980s, the antischistosomal aryl hydantoin Ro 13-3978 (AH01), a close structural analogue of the androgen receptor antagonist nilutamide, was discovered. Administration of 100 mg/kg oral doses of AH01 to mice infected with adult and juvenile *Schistosoma mansoni* produced 95% and 64% total worm burden reductions, confirming its high activity against adult worms, and showing that AH01 is also effective against juvenile infections. AH01 had no measurable interaction with the androgen receptor in a ligand competition assay, but it did block dihydrotestosterone-induced cell proliferation in an androgen-dependent human prostate cancer cell line. For AH01, nilutamide, and three closely related aryl hydantoin derivatives, there was no correlation between antischistosomal activity and androgen receptor interaction.

Schistosomiasis is a neglected but widespread tropical disease caused by infection with parasitic blood flukes. *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* are the most widely distributed species and cause the highest disease burden, particularly in sub-Saharan Africa.^{1–3} Praziquantel (PZ) is the only drug available for treatment of this disease, and it is active against adult schistosomes, but has little activity against the juvenile schistosomula, the young developmental stages of the parasite.^{2,4} Should serious praziquantel drug resistance arise, there are no viable alternatives to this drug. Even so, drug discovery for schistosomiasis has languished.

The introduction of PZ in 1982 likely led to decisions to abandon the development of a number of promising antischistosomal agents that were discovered in the same time period. One of these was Ro 13-3978 (AH01), the lead compound from a series of aryl hydantoins that were investigated in some detail at Hoffmann La-Roche.^{5–8} A number of these aryl hydantoins had high oral efficacy in schistosome animal models,⁸ but they also produced antiandrogenic side effects in the host,⁵ a not unexpected outcome given the close structural similarity of these aryl hydantoins to the antiandrogenic drug nilutamide (Figure 1). We recently showed⁹ that nilutamide, but not the three structurally diverse androgen receptor (AR) antagonists flutamide, bicalutamide, and cyproterone acetate, has antischistosomal activity. As phylogenetic evidence indicates that schistosome species do not appear to have ARs,¹⁰ the data led us to hypothesize that for aryl hydantoins and related heterocycles, the structural requirements for antischistosomal efficacy and AR binding interactions are divergent.

Based on the limited elucidation of the structure-activity-relationship of this aryl hydantoin compound series,⁸ we began to test this hypothesis by the synthesis^{8,11} of AH01, its imino isostere AH02, and two derivatives (AH03 and AH04) incorporating nitrile functional groups (present in bicalutamide and other antiandrogens) known to import high AR binding affinity.¹² We now present our initial characterization of the antischistosomal versus antiandrogenic properties of these four aryl hydantoins.

Interaction of the aryl hydantoins with the AR was characterized by a ligand competition scintillation proximity assay¹³ using a hexahistidine-tagged AR-ligand binding domain (LBD). Briefly, to each well of a 96-well Ni-chelate-coated Flashplate was added 80 μ L of 5 μ M AR-LBD in the assay buffer (25 mM HEPES, pH 7.2, 50 mM NaCl, 0.01% NP-40, 20 μ M dihydrotestosterone [DHT], 2% dimethyl sulfoxide [DMSO]). After 60 min incubation, the protein solution was discarded followed by washes with assay buffer. Then, 40 μ L of serially diluted aryl hydantoins in assay buffer containing 10% DMSO were added into each well followed by addition of 40 μ L of 20 nM [³H]-DHT in assay buffer. The final assay solutions contained 5% DMSO. After sealing the plates and equilibrating for 18 h at 4°C, radio counts were measured and IC₅₀ values were calculated.

As expected, for the homologous DHT competition experiment, we measured a low IC₅₀ value of 76 nM, similar to the previously reported¹³ value of 57 nM. Nilutamide and AH03 had IC₅₀ values of 7.4 and 1.5 μ M, respectively, whereas the other aryl hydantoins had IC₅₀ values > 27 μ M. The binding competition observed for nilutamide and AH03 is consistent with previous data showing that AR ligands with aryl nitro or nitrile functional groups can form ion-dipole interactions with R752 in the AR ligand-binding domain.¹²

To put the AR ligand competition data in context, we assessed the effects of the aryl hydantoins on androgen-dependent LNCaP C-33 human prostate cancer cells.¹⁴ Briefly, LNCaP cells (passage nos. between 39 to 42) were plated at a density of 5×10^4 or 6×10^4 cells/well in 6-well plates for 72 h in a regular RPMI 1640 culture medium containing 5% fetal bovine serum (FBS), which was maintained at 37°C in a humidified atmosphere of 5% CO₂. This was followed by continued cell culture for 48 h in a steroid-reduced medium consisting of phenol red-free RPMI 1640 containing 5% charcoal/dextran-treated FBS (v/v), 2 mM glutamine, and 50 μ g/mL gentamicin. Cells were then exposed for an additional 72 h to 10 μ M test compounds (from 10 mM DMSO stock solutions) with or without DHT (10 nM) after which time cell numbers were counted and cell growth relative to that of control cells was determined.

As expected, DHT significantly induced LNCaP cell proliferation, and this was blocked by the antiandrogen bicalutamide ($P < 0.0001$) (Figure 2). Similarly, AH01 and AH02 blocked DHT-induced LNCaP cell proliferation ($P < 0.001$), but these

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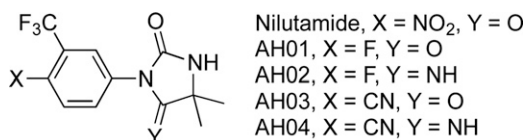


FIGURE 1. Aryl hydantoin structures.

compounds had no effect on basal cell growth. Conversely, nilutamide, AH03, and AH04 alone stimulated LNCaP cell growth ($P < 0.001$), and for nilutamide, addition of DHT decreased ($P < 0.05$) cell proliferation. These data indicate that nilutamide, AH03, and AH04 are AR agonists in this cell line. This is consistent with previous data¹⁵ revealing that nilutamide acts as an AR agonist in LNCaP cells, presumably as a consequence of the T868A mutation in the ligand-binding domain of the AR expressed in this cell line. It is also possible that DHT may trigger a shutoff mechanism after maximal cell proliferation, consistent with previous results that LNCaP cells can respond to androgens in a biphasic concentration-dependent response.^{16,17} For these hydantoin, the apparent discrepancies between the ligand competition scintillation proximity assay and LNCaP cell proliferation data may also be the result of a differential antagonistic effect on tetrameric complex dissociation, AR dimerization, nuclear translocation, and AR interaction with androgen response elements in LNCaP cells.^{18,19} Thus, androgen-sensitive LNCaP C-33 cells may not be the ideal screen to assess the androgenic/antiandrogenic properties of aryl hydantoin.

In vivo (Table 1) antischistosomal activities against *S. mansoni* were assessed as previously described.⁹ Briefly, mice were infected with ~80 *S. mansoni* cercariae on Day 0 followed by administration of single 100 and 200 mg/kg oral doses of test compounds dissolved or suspended in a solubilizing 3% ethanol and 7% Tween 80 vehicle to groups of 4–5 mice on Day 21 post-infection (juvenile stage) and on Day 49 post-infection (adult stage). At 28 days post-treatment, animals were killed by the CO₂ method and dissected to assess total worm reductions.

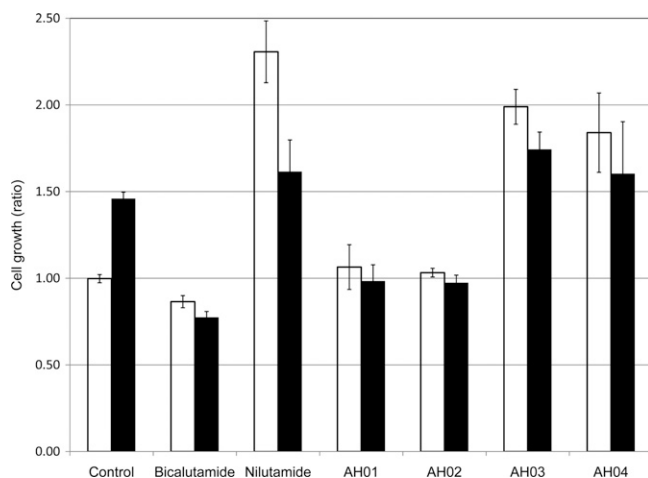


FIGURE 2. Effects of aryl hydantoin derivatives on dihydrotestosterone (DHT)-induced LNCaP cell proliferation. Bars represent the mean \pm SE of the average of 2–4 sets of independent duplicate experiments. Open bars: 10 μ M compound alone; Filled bars: 10 μ M compound + 10 nM DHT.

TABLE 1

Effects of single oral doses of AH01 and AH02 administered to mice harboring 21-day-old juvenile and 49-day-old adult *Schistosoma mansoni* infections

Infection	Compound	Dose (mg/kg)	Mean number of worms (SD)	Total worm burden reduction (%)
Vehicle control 1*	–	–	27.9 (12.5)	–
Vehicle control 2*	–	–	17.4 (5.1)	–
Vehicle control 3*	–	–	24.8 (5.2)	–
Juvenile	AH01	100	6.3 (4.3) [†]	64 [‡]
		200	3.0 (1.9) [§]	88 [‡]
	AH02	100	8.5 (6.8) [†]	51
		200	3.3 (3.3) [¶]	88 [‡]
	Nilutamide	100	–	5
		200	–	22
Adult	AH01	100	1.5 (1.9) [§]	95 [‡]
		100	1.3 (1.3) [†]	93 [‡]
	Nilutamide	100	–	31
		100	–	18

*3% ethanol and 7% Tween 80 solubilizing vehicle.

[†]Versus vehicle control 2.

[‡] $P < 0.05$ from the Kruskal–Wallis test comparing the medians of the responses between the treatment and control groups.

[§]Versus vehicle control 1.

[¶]Versus vehicle control 3.

|| Data from Reference 9.

PZ = praziquantel.

Single 100 mg/kg oral doses of AH01 and AH02 administered to *S. mansoni*-infected mice on Day 49 post-infection (adult worms) afforded respective total worm burden reductions of 95% and 93%, confirming the previously reported⁸ high activities (ED₅₀s of 38 and 37 mg/kg for AH01 and AH02) of these compounds. In this same schistosome mouse model, PZ has an ED₅₀ value of 172 mg/kg.⁴ Similarly, administration of 100 and 200 mg/kg doses of AH01 and AH02 to *S. mansoni*-infected mice on Day 21 post-infection (juvenile worms) afforded respective total worm burden reductions of 64/88 and 51/88%; this clearly shows that aryl hydantoin, unlike PZ, have significant ($P < 0.05$) activity against the juvenile stage of the parasite. In contrast to the high *in vivo* efficacies and lack of *in vivo* toxicity that we observed for AH01 and AH02, administration of single 200 mg/kg oral doses of AH03 and AH04 to *S. mansoni*-infected or uninfected mice led to the death of 60–100% of all animals within 4–5 days post-treatment. The observed toxicity of these two aryl hydantoin, the lack of apparent *in vivo* toxicity for nilutamide in this same experiment,⁹ and the lack of interaction of AH04 with the AR, suggests that the *in vivo* toxicity of these aryl hydantoin derivatives derives in part from androgen-independent mechanisms.

In summary, we confirmed the high antischistosomal efficacy of AH01 and showed that this aryl hydantoin is also effective against juvenile infections. Furthermore, AH01 had no measurable interaction with the AR in a ligand competition assay, but it did block DHT-induced cell proliferation in androgen-dependent human prostate cancer LNCaP cells. For AH01, nilutamide, and the other aryl hydantoin, there was no correlation between antischistosomal activity and androgen receptor interaction.

Although previous investigations⁶ show that AH01 produces schistosome worm lesions similar to those seen after PZ treatment, the molecular mechanism of action of this or related aryl hydantoin is unknown. However, based on our data, their antischistosomal properties do not seem to derive from interactions with the host AR. Finally, it is interesting to note that although schistosome species do not appear to have

ARs,¹⁰ Fantappiè and others²⁰ discovered an expressed schistosome gene encoding the mitochondrial enzyme NADH ubiquinone oxidoreductase subunit 5 by screening a *S. mansoni* cDNA library with a human AR cDNA probe. This mitochondrial enzyme is thought to underlie the observed inhibition of *S. mansoni* growth by high concentrations of testosterone and dehydroepiandrosterone.^{20,21}

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