Macrofilaricidal Benzimidazole–Benzoxaborole Hybrids as an Approach to the Treatment of River Blindness: Part 1. Amide Linked Analogs

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ABSTRACT: A series of benzimidazole–benzoxaborole hybrid molecules linked via an amide linker are described that exhibit good in vitro activity against *Onchocerca volvulus*, a filarial nematode responsible for the disease onchocerciasis, also known as river blindness. The lead identified in this series, 8a (AN8799), was found to have acceptable pharmacokinetic properties to enable evaluation in animal models of human filariasis. Compound 8a was effective in killing *Brugia malayi*, *B. pahangi*, and *Litomosoides sigmodontis* worms present in Mongolian gerbils when dosed subcutaneously as a suspension at 100 mg/kg/day for 14 days but not when dosed orally at 100 mg/kg/day for 28 days. The measurement of plasma levels of 8a at the end of the dosing period and at the time of sacrifice revealed an interesting dependence of activity on the extended exposure for both 8a and the positive control, flubendazole.

KEYWORDS: onchocerciasis, lymphatic filariasis, flubendazole, tubulin, organoboron

Diseases caused by infection of an individual with filarial worms are widespread and of particular concern in the endemic countries of the developing world. Two such diseases are onchocerciasis (river blindness), caused by the parasite *Onchocerca volvulus*, and elephantiasis (lymphatic filariasis, LF), caused by parasites *Wuchereria bancroftii*, *Brugia timori*, and *B. malayi*. These diseases are endemic across Asia (LF) and sub-Saharan Africa (LF and onchocerciasis), with the parasites transmitted via black flies (onchocerciasis) or mosquitoes (lymphatic filariasis). Despite significant and long-term efforts to limit the impact of these parasitic infections on the population through mass drug administration (MDA) programs with microfilaricidal drugs (ivermectin for onchocerciasis; albendazole, ivermectin, and/or diethylcarbamazine for LF), there remains an opportunity to discover, develop, and deliver new drugs that overcome limitations of existing therapies. For example, the current strategy for the treatment of onchocerciasis requires that an infected individual take ivermectin 1–3 times per year for 2–3 years over the lifetime of the adult worms (10–14 years for *O. volvulus* and 6–8 years for *Wuchereria* and *Brugia* spp.), which is logistically challenging in disease endemic areas. Long-term treatment is required because the microfilaricidal drugs kill only the microfilariae of *O. volvulus* or LF; they have little effect on the adult microfilariae. In addition, coinfection of onchocerciasis or LF patients with the eye worm *Loa loa* can limit the utility of treatment with ivermectin due to significant side effects resulting from rapidly killing the *Loa loa* microfilariae.

Flubendazole (1), an inhibitor of tubulin polymerization, has been shown to have the ability to kill adult filarial worms, providing promise that this molecule could have utility in the treatment of onchocerciasis and LF (Figure 1). Despite this promise, flubendazole has several limitations that complicate its potential as a drug for these human infections. First, flubendazole has limited oral bioavailability, primarily a consequence of its poor aqueous solubility. Second, though more selective than other members of the benzimid-
The benzoxaborole core can improve aqueous solubility and oral bioavailability of otherwise poorly soluble molecular frameworks due to the ability of the boron atom to equilibrate between a three coordinate, neutral species (2a) and a four coordinate, negatively charged species (2b) under physiological conditions (Figure 2).

A second potential advantage of the incorporation of the benzoxaborole moiety into a molecule was that the benzoxaborole could engage in unique interactions with the subunits of tubulin in a manner that could impart improved selectivity for inhibition of worm tubulin polymerization relative to mammalian host tubulin. Initial efforts to prepare benzoxaborole–benzimidazole hybrids related to flubendazole focused on simple amides. These compounds were easily prepared from an array of previously described amino- (3), aminooalkyl- (4,5), and carboxy- (10) substituted benzoxaboroles and the corresponding carboxy (6) or amino (11) benzimidazole as depicted in Figure 3.

Table 1. Initial Benzoxaborole–Benzimidazole Amide Leads

| ID  | link atom | n | R     | O. volvulus IC₅₀ (μM) | G2/M arrest IC₅₀ (μM) | MDCK-MDR1 Papp (A-B, ×10⁶ cm/s) |
|-----|-----------|---|-------|-----------------------|-----------------------|----------------------------------|
| 1   | NA        | NA| NA    | 0.004                 | 0.67                  | 15.6                             |
| 7a  | 6         | 0 | 4.55  | >100 NT               | NT                    |                                  |
| 7b  | 6         | 0 | 3,3-Me₂ | >10                  | NT                    |                                  |
| 8a  | 6         | 1 | 3,3-Me₂ | 0.300               | 13                    | 0.8                              |
| 8b  | 3         | 1 | 0.426 | 10                    | NT                    |                                  |
| 8c  | 6         | 1 | 0.421 | >100 NT               | NT                    |                                  |
| 8d  | 5         | 1 | 3,3-Me₂ | 0.042               | 2.54                  | 0.5                              |
| 9a  | 6         | 2 | 3,3-Me₂ | 0.004               | 0.555                 | 0.7                              |
| 12a | 6         | 0 | 3,3-Me₂ | >100 NT             | NT                    |                                  |

**NT = not tested.**
| in vivo model                        | drug treatment                        | worm count median ± SEM (range) | adult worm reduction/animals with no worms | P ≤         | 24 h after last dose | interim necropsy | necropsy |
|------------------------------------|---------------------------------------|---------------------------------|------------------------------------------|-------------|----------------------|------------------|----------|
| B. malayi; necropsy on day 42      | vehicle (SC study), n = 5             | 12 ± 0.73 (11–15)              | 100%/100%                                | 0.0001      | N/A                  | N/A              | N/A      |
|                                    | flubendazole, 10 mg/kg × 5 days, QD, SC, n = 10 | 0 ± 0 (0–0)                   | 100%/100%                                | 0.173       | 0.73                 | NM              | 0.043    |
|                                    | 8a, 150 mg/kg × 14 days, QD, SC (solution), n = 6 | 2 ± 2.14 (0–12)              | 83.3%/50%                                | 0.0622      | 2.47                 | 0.07 (day 28) | 0.01     |
|                                    | 8a, 100 mg/kg × 14 days, QD, SC (suspension), n = 16 | 0 ± 0.19 (0–3)                 | 100%/87.5%                               | 0.0001      | 4.10                 | 5.36 (day 28) | 3.38     |
|                                    | vehicle (PO study), n = 5              | 8 ± 0.81 (5–10)                | 100%/100%                                | N/A         | N/A                  | N/A              | N/A      |
|                                    | 8a, 100 mg/kg × 28 days, QD, PO (suspension), n = 10 | 7 ± 1.38 (4–16)                | 12.5%/0%                                 | 0.9999      | 0.128                | NM              | <LOQ     |
| B. pahangi; necropsy on day 63; n = 5 per group | vehicle                                 | 89 ± 12.79 (64–146)            | 100%/100%                                | N/A         | N/A                  | N/A              | N/A      |
|                                    | flubendazole, 10 mg/kg × 5 days, QD, SC | 0 ± 0 (0–0)                   | 100%/100%                                | 0.0009      | 0.178                | NM              | 0.018    |
|                                    | 8a, 100 mg/kg × 14 days, QD, SC (suspension) | 0 ± 0.33 (0–2)                | 100%/83%                                 | 0.0024      | 5.87                 | NM              | 1.15     |
|                                    | 8a, 100 mg/kg × 28 days, QD, PO (suspension) | 67.5 ± 14.86 (2–114)         | 24%/0%                                   | 0.9999      | 0.13                 | NM              | <LOQ     |
| L. sigmodontis; necropsy on day 63; n = 4 vehicle; n = 6 other groups | vehicle                                 | 8.5 ± 3.28 (5–20)             | N/A                                      | N/A         | N/A                  | N/A              | N/A      |
|                                    | flubendazole, 10 mg/kg × 5 days, QD, SC | 0 ± 0 (0–0)                   | 100%/100%                                | 0.0208      | 0.283                | NM              | 0.040    |
|                                    | 8a, 100 mg/kg × 14 days, QD, SC (suspension) | 0 ± 0.34 (0–2)                | 100%/33.3%                               | 0.1207      | 9.30                 | NM              | 0.069    |
|                                    | 8a, 300 mg/kg × 7 days, QD, SC (suspension) | 0 ± 0 (0–0)                   | 100%/100%                                | 0.0208      | 7.85                 | 10.6 (day 21) | 0.48     |
|                                    | 8a, 100 mg/kg × 28 days, QD, PO (suspension) | 20 ± 6.92 (5–50)              | 13.3%/0%                                 | 0.9999      | 0.210                | 0.006 (day 42) | 0.003    |

“NM = not measured. N/A = not applicable. Statistical significance was tested by Kruskal–Wallis followed by Dunn’s multiple comparisons test.
attention on this substitution pattern.$^{25}$ Increasing the length of the linker between the benzoxaborole and benzimidazole cores as in 9a resulted in a significant increase in \textit{O. volvulus} potency, but also in the G2/M arrest assay we were using as a functional indicator of the interaction with mammalian tubulin. Similarly, changing the point of attachment on the benzoxaborole core from 6- to 5- to 4-ff resulted in a decrease in potency in both the \textit{O. volvulus} and G2/M arrest assays. Lastly, preparation of a reverse amide from the 5-amino benzimidazole and 6-carboxybenzoxaborole (12a) resulted in loss of activity.

We characterized the \textit{in vitro} ADME properties of 8a in preparation for evaluation of this compound in our primary \textit{in vivo} model in gerbils. Metabolic stability of 8a in gerbil microsomes was good (Cl$_{int}$ < 4 μL/min/mg), but it was found to be poorly permeable in an MDR1-MDCK monolayer assay ($P_{app}$ (A-B) = 0.8 × 10$^{-6}$ cm/s).$^{37-39}$ The $P_{app}$ in this assay when the P-glycoprotein (Pgp) efflux inhibitor GF-120918 was added increased to 2.7 × 10$^{-6}$ cm/s, suggesting that the compound was potentially a substrate for this efflux mechanism.$^{40,41}$ Taken together, these data prompted us to explore the activity of 8a following subcutaneous administration to gerbils infected by implantation of adult \textit{Brugia malayi} or \textit{Brugia pahangi} parasites in the peritoneal cavity.$^{42}$ We were pleased to find that 8a was able to kill 100% of both male and female worms in the gerbil peritoneum when dosed subcutaneously at 100 mg/kg/day for 14 days as a suspension in a nonsolubilizing HEC/Tween vehicle. Interestingly, when 8a was dosed subcutaneously at 150 mg/kg/day as a solution in a DMSO/water vehicle, \textit{in vivo} efficacy was substantially reduced. The subsequent assessment of the pharmacokinetics of 8a from these two dosing paradigms provided an interesting observation that we believe to be important in understanding the PK−PD requirements for achieving efficacy in this animal model. In the suspension dose group, plasma levels of 8a were maintained above the \textit{in vitro} IC$_{50}$ in the \textit{O. volvulus} assay (300 nM) for over 42 days after the last dose, whereas in the solution dose group, plasma levels fell below this IC$_{50}$ within a few days after the last dose. We had made the same observation in a positive control group using flubendazole at a subcutaneous dose of 10 mg/kg/day for 5 days in the nonsolubilizing HEC/Tween vehicle, namely, that plasma levels of flubendazole were maintained above the \textit{in vitro} IC$_{50}$ (4 nM) for over 42 days, consistent with data reported in the literature.$^{43}$ As anticipated from our \textit{in vitro} ADME data, 8a was not efficacious when dosed by the oral route (at 100 mg/kg/day for 28 days), and plasma levels of the drug were found to be well below the \textit{in vitro} IC$_{50}$ at all time points. These observations suggest that the efficacy observed in the \textit{in vivo} model was dependent upon long-term exposure of worms to the drug, perhaps a consequence of the "depot-like" properties of the subcutaneous suspension.$^{44}$ We next examined 8a in two additional \textit{in vivo} models, the first where L3 \textit{B. pahangi} larvae were injected into the peritoneum of gerbils and allowed to develop into adult worms$^{45}$ and a second where gerbils were naturally infected by the filarial nematode \textit{Litomosoides sigmodontis}.$^{46,47}$ In both of these models, 8a was administered subcutaneously at 100 mg/kg for 14 days or orally at 100 mg/kg for 28 days as a suspension. As with the adult worm infection model, 8a was quite effective via the subcutaneous route in these additional models but essentially inactive when dosed orally as summarized in Table 2. Additionally, when plasma obtained from treated animals at necropsy was analyzed for 8a, we observed an outcome similar to that observed in the adult implantation model, e.g., that measurable levels were present at this time point in the subcutaneous-dosed groups but not in the orally dosed groups, weeks after administration of the drug. In a final \textit{L. sigmodontis} experiment with 8a, we dosed the compound subcutaneously at 300 mg/kg for 7 days as a suspension. As anticipated, this study demonstrated good activity of 8a, as plasma concentrations of the drug were in excess of the \textit{in vitro} IC$_{50}$ for at least 42 days.

These observations of the dependence of activity on the extended exposure of \textit{B. malayi}, \textit{B. pahangi}, and \textit{L. sigmodontis} worms to the drug (either flubendazole or 8a) were consistent with observations made in an \textit{ex vivo} \textit{B. malayi/pahangi} assay,$^{48}$ namely, that a short (<7 days) exposure of worms to these drugs was not effective in killing the worms. Taken together, these results suggest that the mechanism of action of these benzimidazole drugs (inhibition of tubulin polymerization) requires a long (>28 day) exposure to the drug to be effective.

While we were encouraged by the proof of concept demonstrated by 8a in these \textit{in vivo} models, it was clear that this molecule would not meet our target candidate profile that required an orally active drug candidate.

It has been suggested in the literature that the propensity for Pgp efflux is much greater in compounds containing more than 2−3 hydrogen bond donors (HBDs).$^{39,50}$ Our lead compound

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**Table 3. Analogs Designed To Overcome the Permeability Challenge**

| ID | R1 | R2 | R3 | R4 | R5 | \textit{O. volvulus} IC$_{50}$ (μM) or % inhibition of melting at 1 μM | G2/M IC$_{50}$ (μM) | MDCK-MDR1 $P_{app}$ cm/s $\times 10^6$ |
|----|----|----|----|----|----|---------------------------------|-----------------|----------------------------------|
| 8a | H  | H  | H  | H  | H  | 0.300                           | 13              | 0.8                              |
| 8e | CH$_3$ | H  | H  | H  | H  | 24%$^{25}$                      | >100            | 1.2                              |
| 8f | H  | H  | CH$_3$ | H  | H  | 29%$^{25}$                      | >100            | 1.8                              |
| 8g | H  | CH$_3$ | H  | H  | H  | NT                             | 4.1             | 0.6                              |
| 8h | CH$_3$CH$_2$NMe$_2$ | H  | H  | H  | H  | NT                             | 35              | 0.14                             |
| 8i | H  | H  | H  | F  | H  | 0%$^{25}$                       | 16              | 2.35                             |
| 8j | H  | H  | H  | F  | H  | 0.13                           | 1.26            | 2.83                             |

$^{25}$% of \textit{O. volvulus} larvae that completed molting from L3 to L4 compared to control worms.
(8a) has four potential donors (B−OH, amide NH, benzimidazole NH, and carbamate NH). In order to ameliorate this potential Pgp liability, we prepared and evaluated compounds with fewer HBs (Table 3). As anticipated on the basis of the flubendazole literature, alkylation of the benzimidazole NH (8e, 8f) resulted in loss of activity and also did not improve permeability. Alkylation of the amide nitrogen (8g, 8h) also did not improve permeability. The incorporation of a fluorine substituent on the benzimidazole ring adjacent to the amide (8i, 8j), a strategy that has been shown to “mask” an amide hydrogen bond donor,50,51 did improve the permeability but also affected the potency in both the O. volvulus and G2/M arrest assays. Interestingly, 4-F analog 8i lost activity in the O. volvulus assay, whereas the 7-F analog 8j exhibited greater potency (and hence, poorer selectivity) in both assays.

On the basis of these results, it was clear that more substantial changes needed to be made to the benzoxaborole–benzimidazole hybrids to achieve our objective. The exploration of an additional series of hybrid molecules, most specifically those containing a ketone linker analogous to that found in flubendazole, will be reported in due course.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.9b00396.

Synthesis methods for benzimidazole–benzoxaborole hybrids and methods for testing compounds in larval molt assays and in vivo studies (PDF)

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Notes
The authors declare no competing financial interest.

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(32) Synthesis of compound 8a. Step 1. A mixture of 3-amino-4-(methylamino)benzoic acid (750 mg, 4.81 mmol) and bis(methoxycarbonyl)-2-methylisothiourea (1.86 g, 9.00 mmol) in AcOH (10 mL) was stirred at 80 °C for 20 min and filtered, and the filter cake washed with methanol (20 mL) and ethyl acetate (20 mL), dried in vacuo to give 2-((methoxycarbonyl)amino)-1-methyl-1H-benzimidazole-5-carboxylic acid (80 mg, 32%) as a white solid;1H NMR (400 MHz, DMSO-d6) δ 7.98 (s, 1H), 8.00 (dd, J = 8.4, 1.4, 1H), 7.43 (dd, J = 8.8 Hz, 1H), 3.62 (s, 3H), 3.50 (s, 3H). Step 2. A mixture of 6 (80 mg, 0.32 mmol), 6-(aminomethyl)-3,3-dimethyl-imidazole-5-carboxylic acid (850 mg, 76%) as a white solid;1H NMR (400 MHz, DMSO-d6) δ 7.98 (s, 1H), 7.80 (dd, J = 8.6, 1.4, 1H), 7.50 (dd, J = 8.0 Hz, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.34 (dd, J = 8.0 Hz, 1H), 4.49 (d, J = 4.8 Hz, 2H), 3.70 (s, 3H), 3.60 (s, 3H), 1.40 (s, 6H).
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