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Letter

Macrofilaricidal Benzimidazole−Benzoxaborole Hybrids as an Approach to the Treatment of River Blindness: Part 2. Ketone Linked Analogs

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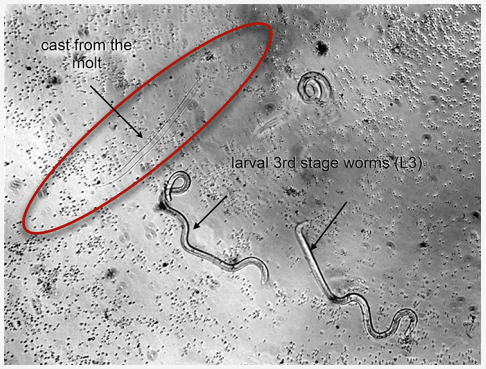
ABSTRACT: The optimization of a series of benzimidazole−benzoxaborole hybrid molecules linked via a ketone that exhibit good activity against *Onchocerca volvulus*, a filarial nematode responsible for the disease onchocerciasis, also known as river blindness, is described. The lead identified in this series, **21** (AN15470), was found to have acceptable pharmacokinetic properties to enable an evaluation following oral dosing in an animal model of onchocerciasis. Compound **21**was effective in killing worms implanted in Mongolian gerbils when dosed orally as a suspension at 100 mg/kg/day for 14 days but not when dosed orally at 100 mg/kg/day for 7 days.



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

Previous work from our laboratory has described the discovery and development of a series of benzimidazole−benzoxaborole hybrid molecules that were effective in inhibiting the molting *in vitro* of the parasitic filarial worm *Onchocerca volvulus* as well as killing *Brugia malayi* and *B. pahangi* adult worms *in vivo*.[1] *O. volvulus*, *Wuchereria bancrofti*, *B. malayi*, and *B. timori* are responsible for the diseases known as river blindness (onchocerciasis) and elephantiasis (lymphatic filariasis, LF) that are endemic in the developing world.[2] Despite significant and long-term efforts to limit the impact of these parasitic infections on the population through mass drug administration (MDA) programs including ivermectin, albendazole, and/or diethylcarbamazine,[3−6] there remains a need to identify new treatments that can kill specifically adult worms using a short course of treatment and not just microfilariae.[5−8] 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.[9,10]

The strategy of our program was based on the known benzimidazole carbamate flubendazole (**3**), an inhibitor of tubulin polymerization that had shown antifilarial activity.[11−14] Flubendazole (**3**) is a member of a larger class of benzimidazole carbamate tubulin polymerization inhibitors and contains a 4-fluorophenyl substituent linked to the benzimidazole core via a ketone at C(6). One of the main limitations of flubendazole as an antifilarial is the limited bioavailability of this molecule, most likely a consequence of poor aqueous solubility.[15,16] We sought to overcome this limitation by utilization of the benzoxaborole in place of the 4-fluorophenyl substituent. This boron-containing heterocycle can exist in equilibrium between a trigonal, neutral boron atom (**1**) and a tetrahedral, negatively charged boron atom (**2**) under physiologically relevant conditions, which have improved aqueous solubility (Figure 1).[17−28]

In addition, flubendazole has been found to be an aneugen in both *in vitro* and *in vivo* micronucleus tests, although it has been argued that the lack of clastogenicity of flubendazole in these tests will limit the risk of carcinogenicity to patients.[29] However, the metabolism of flubendazole by the reduction of the ketone leads to short-lived clastogenic metabolites at low levels that may pose a minimal risk.[29] Though, extensive

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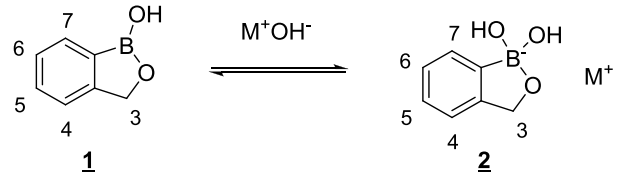


Figure 1. Equilibrium between trigonal and tetrahedral boron in the benzoxaboroles.

metabolite ID studies on our lead compounds, particularly compound **21**, have not observed a significant metabolism of these compounds in either *in vitro* or *in vivo* experiments, suggesting that this risk may be reduced in the benzoxaborole–benzimidazole hybrids (*vide infra*).

The lead compound identified in our earlier work, AN8799 (**4**), was a hybrid molecule wherein the benzimidazole and benzoxaborole moieties were connected via an amide linker (Figure 2).

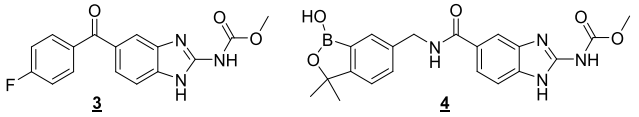


Figure 2. Structures of flubendazole (**3**) and AN8799 (**4**).

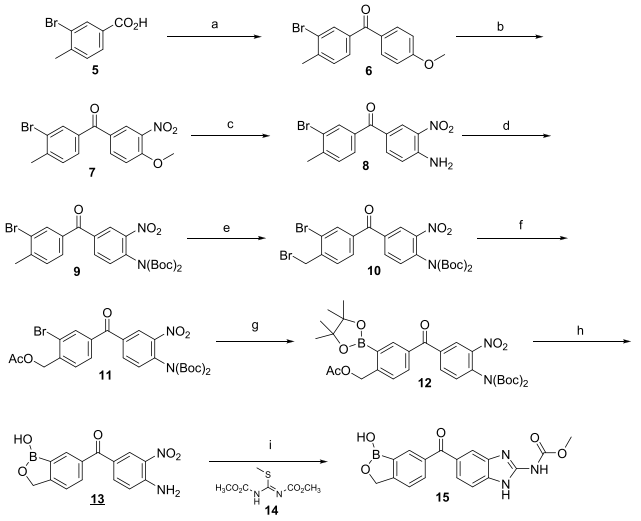
While this compound was found to exhibit good *in vitro* antiparasitic activity and selectivity relative to the effects on mammalian cells, we discovered that it was only active in *in vivo* models of LF following subcutaneous administration. This limitation was understood on the basis of the pharmacokinetic properties of **4**, specifically that it was subject to efflux via the P-glycoprotein transporter (Pgp), limiting its oral bioavailability. We describe here subsequent efforts to explore ketone-linked benzoxaborole–benzimidazole hybrids designed to overcome Pgp efflux.

The synthesis of ketone-linked benzimidazole–benzoxaborole hybrids was based on the well-known route to flubendazole and close analogs.[30] For example, the optimized route to the 6-oxobenzoxaborole isomer is shown in Scheme 1.

Starting with 3-bromo-4-methylbenzoic acid (**5**), the conversion to the acid chloride followed by Friedel–Crafts acylation of anisole afforded the diaryl ketone (**6**). Nitration, followed by displacement of the 4-methoxy substituent, provided the nitroaniline (**8**), which was protected as the di-*tert*-butyl carbonate (**9**). Benzylic bromination with NBS/AIBN followed by displacement with sodium acetate gave the key intermediate aryl bromide (**11**), which was converted to the benzoxaborole (**13**) via palladium mediated borylation, hydrolysis, and ring closure. Reduction of the nitro group and condensation with the isothiourea derivative (**14**) completed the synthesis of the ketone hybrid linked via C(5) of the benzimidazole and C(6) of the benzoxaborole (**15**). Additional benzoxaborole isomers were prepared in similar sequences starting from the appropriate bromotoluic acids shown in Scheme 2.

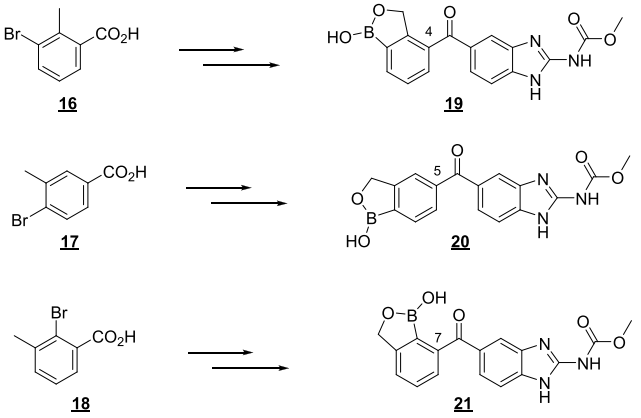
Additionally, hybrid molecules where the benzimidazole ring was linked ring via C(4), C(5), or C(7) of the benzoxaborole ring (**19**–**21**) were prepared in an analogous manner from appropriately substituted bromotoluic acids (**16**–**18**). Also prepared were several compounds containing substituents on the benzoxborole ring (**22**–**30**) and two analogs where the linker between the benzimidazole and benzoxaborole ring was

Scheme 1. Synthesis of the 6-Oxobenzoxaborole–Benzimidazole Hybrid Molecule[a]



[a]Reagents and conditions: (a) $SOCl_2$, DCM, DMF (cat.) and then anisole, $AlCl_3$, DCM, 60%; (b) HNO_3, H_2SO_4, DCM, 55%; (c) NH_3, iPrOH, 100 °C, sealed tube, 52%; (d) Boc_2O, pyridine, THF, 62%; (e) NBS, AIBN, CCl_4, 87%; (f) NaOAc, DMF, 23%; (g) B_2pin_2, KOAc, Pd(dppf)Cl_2, 1,4-dioxane, 97%; (h) NaOH and MeOH and then HCl, H_2O, THF, 45%; (i) Fe(0), NH_4Cl, EtOAc, H_2O and then **14**, AcOH, 29%.

Scheme 2. Synthetic Approach to 4-, 5-, and 7-Oxobenzoxaborole–Benzimidazole Hybrids



extended by two or three atoms (**31**, **32**), as we had observed that ether, thioether, or simple alkyl linkers had provided very potent macrofilaricides (unpublished data).

As summarized in Table 1, we were encouraged to find that the initial ketone analogs prepared, namely, **15** (6-linked) and **20** (5-linked), were quite active in an *O. volvulus* molting assay we used as a primary indicator of antiparasitic activity[31,32] and were only weakly active in a G2/M arrest,[33] indicative of the interaction with mammalian tubulin. As described above, our hypothesis was to reduce Pgp efflux liability through elimination of the hydrogen-bond donor associated with the N–H of the amide linker, as this has been shown in the literature to be a contributor to recognition by Pgp.[34,35] Consequently, we were encouraged to find that both **15** and **20** exhibited improvement in permeability in an MDCK-MDR1 cell monolayer assay, but we were disappointed to find that this did not translate into significantly improved exposure following

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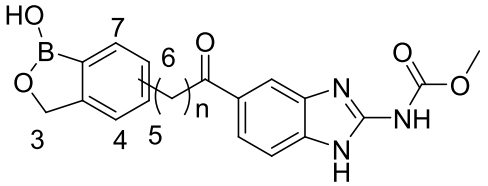
Table 1. *In Vitro* Data for Ketone-Linked Benzoxaborole−Benzimidazole Analogs[a]



ID	link atom	*n*	R	*O. volvulus* IC_{50} (μM)	G2/M IC_{50} (μM)	Cl_{int} in microsomes (μL/min/mg) gerbil	Cl_{int} in microsomes (μL/min/mg) human	MDCK-MDR1 P_{app} (A-B, ×10^6 cm/s)
3	NA, flubendazole			0.004	0.67	<4	NT	15.6
15	6	0		0.118	12.3	8.25	<4	6.0
19	4	0		1.59	>100	NT	5.56	28.3
20	5	0		0.112	9.8	11.7	<4	11.7
21	7	0		0.10	38.4	NT	5.90	56.6
22	5	0	3,3-Me_2	0.01	0.28	NT	NT	26.2
23	6	0	3,3-Me_2	>1.0 (note 1)	>100	NT	NT	20.7
24	6	0	3-Me (*R**)	>1.0 (note 2)	>100	NT	NT	NT
25	6	0	3-Me (*S**)	0.04	>100	<4	<4	15.5
26	7	0	4-F	0.02	30.3	11.6	11.1	21.7
27	7	0	5-F	0.27	22.0	<4	<4	32.0
28	7	0	6-F	1.31	>100	NT	NT	NT
29	7	0	3-Me (*R**)	0.10	5.6	<4	<4	57.2
30	7	0	3-Me (*S**)	0.11	12.2	<4	<4	34.3
31	7	0	4-Cl	0.03	11.6	<4	<4	28.0
32	6	2	3,3-Me_2	0.29	0.46	26.7	7.63	16.0
33	6	3	3,3-Me_2	NT	0.02	NT	NT	NT

[a]Note 1: 0% inhibition at 1 μM. Note 2: 2% inhibition at 1 μM.

oral administration to gerbils (Table 2). Metabolic stability in both gerbil and human microsomes was good, with Cl_{int} values < 15 μL/min/mg for both compounds, suggesting that rapid metabolism was not responsible for the poor exposure. We have observed in other benzoxaborole classes that 3,3-dimethyl substituted analogs frequently provide improved pharmacokinetics over 3,3-unsubstituted analogs;[23] hence, we prepared these analogs of **15** and **20**. We were disappointed to find that the 5-linked analog **22** was poorly selective and the 6-linked analog **23** was only weakly active. Encouragingly, both compounds exhibited significantly improved permeability, suggesting that continued effort in the ketone-linked series is warranted. As had been observed in the amide-linked series, increasing the length of the linker between the benzoxaborole and benzimidazole cores as in **32** and **33** resulted in high potency in the G2/M arrest assay, rendering these compounds unattractive to progress forward due to the lack of selectivity.

Table 2. Pharmacokinetic Properties of Benzoxaborole−Benzimidazole Analogs in Mongolian Gerbils

compound	dose	route	C_{max} (μg/mL)	AUC_{0-24} (h·μg/mL)	bioavailability (%F)
15	2	IV	2.26	0.88	
15	10	PO	0.27	0.79	18
20	2	IV	2.00	1.16	
20	10	PO	0.48	0.99	17
21	2	IV	1.56	5.95	
21	10	PO	5.75	61.7	~100
26	2	IV	10.9	18.6	
26	10	PO	1.69	23.5	24
30	2	IV	7.58	14.6	
30	10	PO	14.0	141	~100

Moving the ketone linker to the 4-position of the benzoxaborole ring provided **19**, which was less potent in the *O. volvulus* molting assay but exhibited good permeability. Much more encouraging data was obtained for the hybrid linked via the 7-position of the benzoxaborole, **21**, which showed very good activity in the *O. volvulus* molting assay (IC_{50} = 100 nM), low activity in the G2/M arrest assay (IC_{50} > 38 μM), and very high permeability (P_{app} = 56.6 × 10^{-6} cm/s). We hypothesize that the high permeability of this isomer is the result of an intramolecular hydrogen bond between the B−OH and ketone carbonyl, which effectively masks this hydrogen bond donor. This hypothesis is supported by the observation that other 7-linked ketones (**26**−**31**) prepared in the project showed similarly high permeability.

Most importantly, **21** was found to exhibit very good exposure when dosed orally to gerbils (Table 3). We observed that **21** provided very high blood concentrations that were

Table 3. Pharmacokinetic Properties of 21 in Mongolian Gerbils by IV, PO, and SC Routes[a]

route	dose	C_{max} (μg/mL)	Cl_{int} (mL/h/kg)	V_{dss} (mL/kg)	$AUC_{0\text{-}last}$ (h·μg/mL)	%F
IV	2	1.56	289	3188	5.95	NA
SC	10	3.13	NA	NA	35.7	100
PO	10	5.75	NA	NA	61.7	100
PO	30	17.2	NA	NA	196	NC
PO	60	26.3	NA	NA	361	NC
PO	100	34.5	NA	NA	479	NC

[a]NA = Not applicable. NC = Not calculated.

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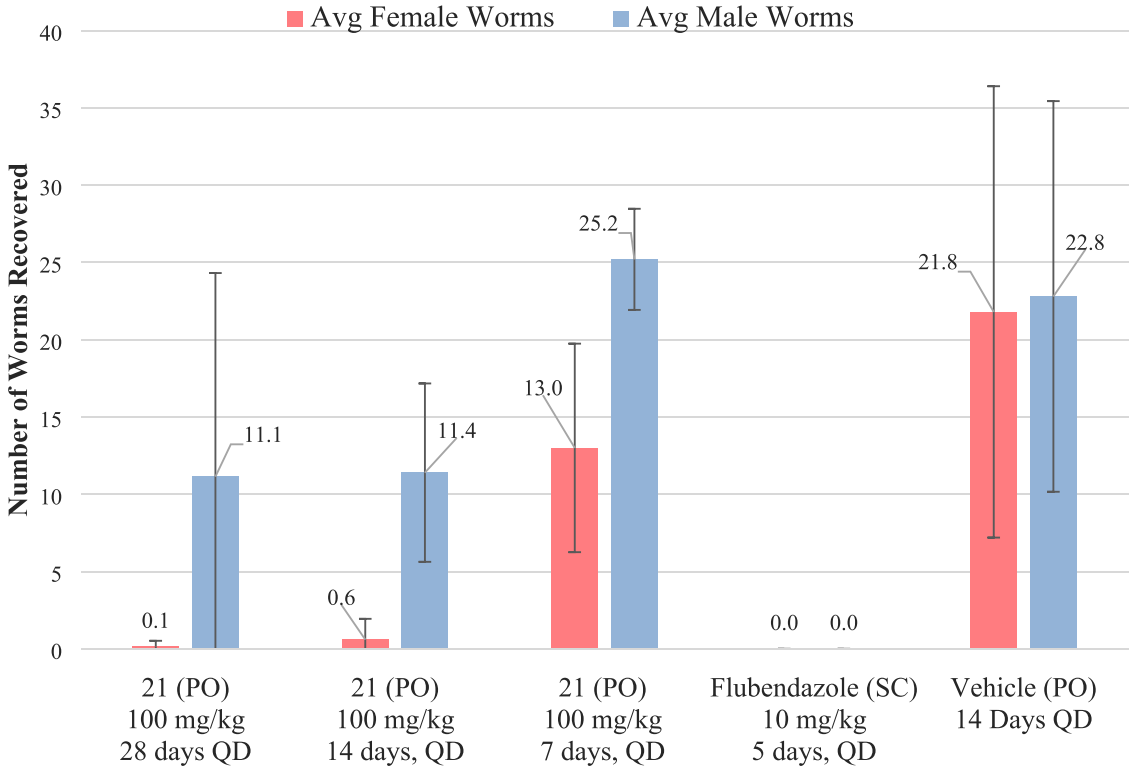

Figure 3. *In vivo* efficacy of **21** in a *Brugia pahangi* model.

maintained for several hours following oral dosing and that the exposure was dose proportional up to 100 mg/kg. In addition, the concentrations achieved at these doses were well in excess of the *O. volvulus* IC_{50}, a requirement for *in vivo* activity that we had hypothesized on the basis of earlier work in the amide-linked series. Finally, we observed that once daily dosing of **21** to gerbils over 7 days was well tolerated and no accumulation of the compound was observed over this time period, providing confidence to progress this compound to a series of *in vivo* efficacy assays in infected gerbils.

For our *in vivo* model, gerbils were infected by injecting *B. pahangi* third-stage larvae (L3) that were subsequently allowed to develop to adult worms over several months. Once stable infections had been established, **21** was administered as a suspension via once-daily oral gavage at a dose of 100 mg/kg for 7, 14, or 28 days. All animals were maintained until day 63 after the start of dosing, at which time adult worms were recovered from the peritoneal cavity and microfilariae were quantified in blood samples. Flubendazole (5 mg/kg, SC × 5 days) was included as a positive control in this study.

We were encouraged to find that **21**, when dosed for 14 or 28 days, was 99% effective in killing female worms, although the effects on the male worms were less impressive. Interestingly, very little effect was observed when **21** was dosed for only 7 days (Figure 3). As anticipated, flubendazole given SC worked as expected, with no worms recovered from this positive control group. Differential sensitivity of male and female *Brugia* was also observed when infected jirds were given oral doses of an amorphous solid dispersion formulation (ASD) of flubendazole.[36] Female worms were reduced by 52−93% in animals dosed with 0.2−15 mg/kg ASD of flubendazole for 5 days. Similar to the ASD of flubendazole, the extended exposure of worms to a tubulin inhibitor such as **21** may cause more extensive damage to female worms than male worms.

To further explore this possibility, we progressed **21** to a more extensive evaluation in additional *in vivo* efficacy models that included the evaluation of worm damage through transmission electron microscopy (TEM) studies of worms recovered from jirds. These studies 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.9b00397.

Synthesis methods for benzimidazole−benzoxiborole hybrids and methods for testing compounds in larval molt assays and *in vivo* studies (PDF)

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Author Contributions

R.T.J., D.S.C., Y.R.F., and J.A.S. wrote the manuscript and contributed equally to the design and execution of the project. D.S.C., T.A., R.T.J., and J.J.P. designed and coordinated the synthesis of the compounds. R.T.J., Y.R.F., C.S.L., E.E.E., F.R., J.J.P., R.S., J.M., J.A.S., and S.L. provided scientific leadership and management of the project. P.W.B. designed, coordinated, and interpreted the *in vitro* and *in vivo* pharmacokinetics studies. C.F., C.A.B., K.C.L., B.M.S., and N.T. conducted the *in vitro* and *in vivo* biological assays, which were designed and coordinated by Y.R.F., C.S.L., F.R., S.L., and J.A.S. The manuscript was edited by R.T.J., D.S.C., Y.R.F., P.W.B., S.L., C.A.B., and J.A.S.

Notes

The authors declare no competing financial interest.

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