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

David S. Carter, Robert T. Jacobs,* Yvonne R. Freund, Pamela W. Berry, Tsutomu Akama, Eric E. Easom, Christopher S. Lunde, Fernando Rock, Rianna Stefanakis, James McKerrow, Chelsea Fischer, Christina A. Bulman, Kee Chong Lim, Brian M. Suzuki, Nancy Tricoche, Judy A. Sakanari, Sara Lustigman, and Jacob J. Plattner

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. 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. 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. 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.5,10

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...
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 1. Equilibrium between trigonal and tetrahedral boron in the benzoxaboroles.](image1.png)

![Figure 2. Structures of flubendazole (3) and AN8799 (4).](image2.png)

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 benzoxaborole ring (22−30) and two analogs where the linker between the benzimidazole and benzoxaborole ring was extended by two or three atoms (31, 32), as we had observed that ether, thioether, or simple alkyl linkers had provided very potent macroflaricides (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 improved permeability in an MDCK-MDR1 cell monolayer assay, but we were disappointed to find that this did not translate into significantly improved exposure following

---

**Scheme 1. Synthesis of the 6-Oxobenzoxaborole−Benzimidazole Hybrid Molecule**

![Scheme 1](image3.png)

Reagents and conditions: (a) SOCl₂, DCM, DMF (cat.) and then anisole, AlCl₃, DCM, 60%; (b) HNO₃, H₂SO₄, DCM, 55%; (c) NH₃, iPrOH, 100 °C, sealed tube, 52%; (d) Boc₂O, pyridine, THF, 62%; (e) NBS, AIBN, CCl₄, 87%; (f) NaOAc, DMF, 23%; (g) Bpin₂, KOAc, Pd(dppf)Cl₂, 1,4-dioxane, 97%; (h) NaOH and MeOH and then HCl, H₂O, THF, 45%; (i) Fe(0), NH₄Cl, EtOAc, H₂O and then 14, AcOH, 29%.

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

![Scheme 2](image4.png)
Table 1. In Vitro Data for Ketone-Linked Benzoxaborole–Benzimidazole Analogs

| ID | link atom | n | R | O. volvulus IC_{50} (μM) | G2/M IC_{50} (μM) | Cl_{int} in microsomes (μL/min/mg) | gerbil | human | MDCK-MDR1 P_{app} (A-B, ×10^6 cm/s) |
|----|-----------|---|---|--------------------------|------------------|-----------------------------------|-------|-------|-----------------------------|
| 3  | NA, flubendazole | 6 | 0 | 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-Me₂ | 0.01 | 0.28 | NT | 26.2 |
| 23 | 6 | 0 | 3,3-Me₂ | >1.0 (note 1) | >100 | NT | NT |
| 24 | 6 | 0 | 3-Me (R*) | >1.0 (note 1) | >100 | NT | NT |
| 25 | 6 | 0 | 3-Me (S*) | 0.04 | >100 | <4 | 15.5 |
| 26 | 7 | 0 | 4-F | 0.02 | 30.3 | 11.6 | 21.7 |
| 27 | 7 | 0 | 5-F | 0.27 | 22.0 | <4 | 3.20 |
| 28 | 7 | 0 | 6-F | 1.31 | >100 | NT | NT |
| 29 | 7 | 0 | 3-Me (R*) | 0.10 | 5.6 | <4 | 57.2 |
| 30 | 7 | 0 | 3-Me (S*) | 0.11 | 12.2 | <4 | 34.3 |
| 31 | 7 | 0 | 4-Cl | 0.03 | 11.6 | <4 | 28.0 |
| 32 | 6 | 2 | 4-Me₂ | 0.29 | 0.46 | 26.7 | 7.63 |
| 33 | 6 | 3 | 3,3-Me₂ | NT | 0.02 | NT | NT |

<sup>Note 1: 0% inhibition at 1 μM. Note 2: 2% inhibition at 1 μM.</sup>

<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;<sup>15</sup> 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.

Moving the ketone linker to the 4-position of the benzoxaborole ring provided 19, which was less potent in the <i>O. volvulus</i> 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 <i>O. volvulus</i> molting assay (IC_{50} = 100 nM), low activity in the G2/M arrest assay (IC_{50} > 38 μM), and very high permeability (<i>P_{app}</i> = 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 2. Pharmacokinetic Properties of Benzoxaborole–Benzimidazole Analogs in Mongolian Gerbils

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

<sup>NA = Not applicable. NC = Not calculated.</sup>

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

| route | dose | C_{max} (μg/mL) | Cl_{int} (mL/h/kg) | V_{ss} (mL/kg) | AUC_{0-24} (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 |

<sup>NA = Not applicable. NC = Not calculated.</sup>
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–benzoxaborole hybrids and methods for testing compounds in larval molt assays and *in vivo* studies (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
Robert T. Jacobs — Anacor Pharmaceuticals, Inc., Palo Alto, California 94303, United States; orcid.org/0000-0001-9669-2862; Email: rtjacobs7158@gmail.com

**Authors**
David S. Carter — Anacor Pharmaceuticals, Inc., Palo Alto, California 94303, United States
Yvonne R. Freund — Anacor Pharmaceuticals, Inc., Palo Alto, California 94303, United States
Pamela W. Berry — Anacor Pharmaceuticals, Inc., Palo Alto, California 94303, United States
Tsutomu Akama — Anacor Pharmaceuticals, Inc., Palo Alto, California 94303, United States
Eric E. Easom — Anacor Pharmaceuticals, Inc., Palo Alto, California 94303, United States
Christopher S. Lunde — Anacor Pharmaceuticals, Inc., Palo Alto, California 94303, United States

**Figure 3. In vivo efficacy of 21 in a *Brugia pahangi* model.**
Complete contact information is available at:

Foundation for their support and guidance, Jason Zhang and Elliott and Ken Duncan of the Bill & Melinda Gates California San Francisco (OPP1017584). We thank Richard The authors would like to thank the Bill & Melinda Gates The authors declare no competing Notes

Author Contributions
J.J.P., R.S., J.M., J.A.S., and S.L. provided scienti... 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

S.C.A.B., and J.A.S.

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.

ACKNOWLEDGMENTS

The authors would like to thank the Bill & Melinda Gates Foundation for funding of this program through awards to Anacor (Contract Number 23629) and the University of California San Francisco (OPP1017584). We thank Richard Elliott and Ken Duncan of the Bill & Melinda Gates Foundation for their support and guidance, Jason Zhang and his team at Acme Bioscience, Inc. for their contributions to the medicinal chemistry, and Chris Franklin for his help with the graphics.

REFERENCES

(1) Akama, T., Freudin, Y. R., Berry, P. W., Carter, D. S., Carter, D. S., Easom, E. E., Jarnagin, K., Lunde, C. S., Plattner, J. J., Rock, F., Stefanakis, R., Fischer, C., Bulman, C., Lim, K. C., Suzuki, B. M., Tricoche, N., Mansour, A., DiCosty, U., McCall, S., Carson, B., McCall, J. W., McKerrow, J. H., Hübner, M. P., Specht, S., Hoerauf, A., Lustigman, S., Sakarani, J., and Jacobs, R. T. (2020) Macrofilaridal Benznidazole-Benzoxaborole Hybrids as an Approach to the Treatment of River Blindness: Part I. Amide Linked Analogs ACS Inf. Dis., in press, DOI: 10.1021/acsinfecdis.9b00396.
(2) Simonsen, P. E. F., Fischer, P. U., Hoerauf, A., and Weil, G. J. (2014) The Filariases. In Manson’s Tropical Diseases (Farrar, J. H., Hotez, P., Junghanss, T., Kang, G., Lalloo, D., and White, N. J., Eds.) 23rd ed., pp 737–765, Elsevier Saunders.
(3) Ichimori, K., King, J. D., Engels, D., Yajima, A., Mikhailov, A., Lammie, P., and Ottesen, E. A. (2014) Global programme to eliminate lymphatic filariasis: the processes underlying programme success. PLoS Neglected Trop. Dis. 8 (12), No. e0003328.
(4) Hooper, P. J., Bradley, M. H., Biswas, G., and Ottesen, E. A. (2009) The Global Programme to Eliminate Lymphatic Filariasis: health impact during its first 8 years (2000–2007). Ann. Trop. Med. Parasitol. 103 (Suppl 1), 17–21.
(5) Thomsen, E. K., Sanuku, N., Baea, M., Sotafon, S., Maki, E., Lombore, B., Schmidt, M. S., Siba, P. M., Weil, G. J., Kazura, J. W., Fleckenstein, L. L., and King, C. L. (2016) Efficacy, safety, and pharmacokinetics of coadministered diethylcarbamazine, albendazole, and ivermectin for treatment of Bancroftian filariasis. Clin. Infect. Dis. 62, 334–341.
(6) Fischer, P. U., King, C. I., Jacobson, J. A., and Weil, G. J. (2017) Potential value of triple drug therapy with ivermectin, diethylcarbamazine, and albendazole (IDA) to accelerate elimination of lymphatic filariasis and onchocerciasis in Africa. PLoS Neglected Trop. Dis. 11 (1). No. e0005163.
(7) Geary, T. G. (2005) Ivermectin 20 years on: maturation of a wonder drug. Trends Parasitol. 21 (11), 530–532.
(8) Goa, K. L., McTavish, D., and Clissold, S. P. (1991) Ivermectin. A review of its antifilarial activity, pharmacokinetic properties and clinical efficacy in onchocerciasis. Drugs 42 (4), 640–658.
(9) Kamano, J., Pion, S. D., Tejioke, M. C., Twum-Danso, N. A., Thylefors, B., and Boussinesq, M. (2007) Randomized, controlled, double-blind trial with ivermectin on Loa loa microfilaraemia: efficacy of a low dose (approximately 25 microg/kg) versus current standard dose (150 microg/kg). Trans. R. Soc. Trop. Med. Hyg. 101 (8), 777–785.
(10) Gardon, J., Gardon-Wendel, N., Demanga, N., Kamnoj, J., Chippaux, J. P., and Boussinesq, M. (1997) Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for Loa loa infection. Lancet 350 (9070), 18–22.
(11) Mackenzie, C. D., and Geary, T. G. (2011) Flubendazole: a candidate macrofilaricide for lymphatic filariasis and onchocerciasis field programs. Expert Rev. Anti-Infect. Ther. 9 (5), 497–501.
(12) Dominguez-Vazquez, A., Taylor, H. R., Greene, B. M., Ruvalcaba-Macias, A. M., Rivas-Alcala, A. R., Murphy, R. P., and Beltran-Hernandez, F. (1983) Comparison of flubendazole and diethylcarbamazine in treatment of onchocerciasis. Lancet 321 (8317), 139–143.
(13) Denham, D. A., and Brandt, E. (1980) Chemoprophylactic activity of flubendazole against adult Brugia pahangi transplanted into the peritoneal cavity of jirds. J. Parasitol. 66 (6), 933–934.
(14) Denham, D. A., Samad, R., Cho, S. Y., Suswillo, R. R., and Skippins, S. C. (1979) The anthelminthic effects of flubendazole on Brugia pahangi. Trans. R. Soc. Trop. Med. Hyg. 73 (6), 673–676.
(15) Lanusse, C. E., and Prichard, R. K. (1993) Clinical pharmacokinetics and metabolism of benzimidazole anthelmintics in ruminants. Drug Metab. Rev. 25 (3), 235–279.
(16) Ceballos, L., Moreno, L., Torrado, J. J., Lanusse, C., and Alvarez, L. (2012) Exploring flubendazole formulations for use in sheep. Pharmacokinetic evaluation of a cyclodextrin-based solution. BMC Vet. Res. 8, 71.
(17) Van Bocxlaer, K., Gaukel, E., Hauser, D., Park, S. H., Schock, S., Yardley, V., Randolph, R., Plattner, J. J., Merchant, T., Croft, S. L., Jacobs, R. T., and Wring, S. A. (2018) Topical Treatment for
Cutaneous Leishmaniasis: Dermato-Pharmacokinetic Lead Optimization of Benzoxaboroles. Antimicrob. Agents Chemother. 62 (5), e02419-17.

(18) Akama, T., Zhang, Y. K., Freund, Y. R., Berry, P., Lee, J., Easom, E. E., Jacobs, R. T., Plattner, J. J., Witty, M. J., Peter, R., Rowan, T. G., Gillingwater, K., Brun, R., Nare, B., Mercer, L., Xu, M., Wang, J., and Liang, H. (2018) Identification of a 4-fluorobenzyl 1-valinate amide benzoxaborole (AN11736) as a potential development candidate for the treatment of Animal African Trypanosomiasis (AAT). Bioorg. Med. Chem. Lett. 28 (1), 6–10.

(19) Zhang, Y. K., Plattner, J. J., Easom, E. E., Jacobs, R. T., Guo, D., Freund, Y. R., Berry, P., Ciarravino, V., Erve, J. C. L., Rosenthal, P. J., Campo, B., Gamo, F. J., Sanz, L. M., and Cao, J. (2017) Benzoxaborole Antimalarial Agents. Part S. Lead Optimization of Novel Amide Pyrazinyloxy Benzoxaboroles and Identification of a Preclinical Candidate. J. Med. Chem. 60 (13), 5889–5908.

(20) Zhang, Y. K., Plattner, J. J., Easom, E. E., Zhou, Y., Akama, T., Bu, W., White, W. H., Defauw, J. M., Winkle, J. R., Balko, T. W., Guo, S., Xu, J., Cao, J., and Zou, W. (2015) Discovery of an orally bioavailable isoxazoline benzoxaborole (AN8030) as a long acting animal ectoparasiticide. Bioorg. Med. Chem. Lett. 25 (23), 5589–5593.

(21) Adamczyk-Wozniak, A., Borys, K. M., and Sporzyński, A. (2015) Recent Developments in the Chemistry and Biological Applications of Benzoxaboroles. Chem. Rev. 115 (11), 5224–5247.

(22) Jacobs, R. T., Plattner, J. J., and Keenan, M. (2011) Boron-based drugs as antiprotozoals. Curr. Opin. Infect. Dis. 24 (6), 586–592.

(23) Jacobs, R. T., Nare, B., Wring, S. A., Orr, M. D., Chen, D., Sligar, J. M., Jenkins, M. X., Noe, R. A., Bowling, T. S., Mercer, L. T., Rewerts, C., Gaukel, E., Owens, J., Parham, R., Randolph, R., Beaudet, B., Bacchi, C. J., Yarlett, N., Plattner, J. J., Freund, Y., Ding, C., Akama, T., Zhang, Y. K., Brun, R., Kaiser, M., Scandale, I., and Don, R. (2011) SCYX-7158, an orally-active benzoxaborole for the treatment of stage 2 human African trypanosomiasis. PLoS Neglected Trop. Dis. 5 (6), No. e001151.

(24) Nare, B., Wring, S., Bacchi, C., Beaudet, B., Bowling, T., Brun, R., Chen, D., Ding, C., Freund, Y., Gaukel, E., Hussain, A., Jarnagin, K., Jenkins, M., Kaiser, M., Mercer, L., Mejia, E., Noe, A., Orr, M., Parham, R., Plattner, J. J., Randolph, R., Rattendi, D., Rewerts, C., Sligar, J., Yarlett, N., Don, R., and Jacobs, R. (2010) Discovery of novel orally bioavailable oxaborole 1-carboxamides that demonstrate cure in a murine model of late-stage central nervous system african trypanosomiasis. Antimicrob. Agents Chemother. 54 (10), 4379–4388.

(25) Adamczyk-Wozniak, A., Cyran, M. K., Jakubczyk, M., Klimentowska, P., Koll, A., Kolodziejczyk, J., Pojmań, G., Zubrowska, A., Zukowska, G. Z., and Sporzyński, A. (2010) Influence of the substituents on the structure and properties of benzoxaboroles. J. Phys. Chem. A 114 (6), 2324–2330.

(26) Hui, X., Baker, S. J., Wester, R. C., Barbadillo, S., Cashmore, A. K., Sanders, V., Hold, K. M., Akama, T., Zhang, Y. K., Plattner, J. J., and Maibach, H. I. (2007) Vitro penetration of a novel oxaborole antifungal (AN2690) into the human nail plate. J. Pharm. Sci. 96 (10), 2622–2631.

(27) Baker, S. J., Zhang, Y. K., Akama, T., Lau, A., Zhou, H., Hernandez, V., Mao, W., Alley, M. R., Sanders, V., and Plattner, J. J. (2006) Discovery of a new boron-containing antifungal agent, 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690), for the potential treatment of onychomycosis. J. Med. Chem. 49 (15), 4447–4450.

(28) Baker, S. J., Akama, T., Zhang, Y. K., Sauro, V., Pandit, C., Singh, R., Kully, M., Khan, J., Plattner, J. J., Benkovic, S. J., Lee, V., and Maples, K. R. (2006) Identification of a novel boron-containing antibacterial agent (AN0128) with anti-inflammatory activity, for the potential treatment of cutaneous diseases. Bioorg. Med. Chem. Lett. 16 (23), 5963–5967.

(29) Tweats, D. J., Johnson, G. E., Scandale, I., Whitwell, J., and Evans, D. B. (2016) Genotoxicity of flubendazole and the impact of a new formulation on in vivo anaeugenicity. Mutagenesis 31, 309–321.

(30) Van Gelder, J. L. R., Frans, L., and Raeymaekers, A. H. (1972) Benzimidazole Carbamates. U.S. Patent 3,657,267.

(31) Gloeckner, C., Garner, A. L., Mersha, F., Oksov, Y., Tricoche, N., Eubanks, L. M., Lustgman, S., Kaufmann, G. F., and Janda, K. D. (2010) Repositioning of an existing drug for the neglected tropical disease Onchocerciasis. Proc. Natl. Acad. Sci. U. S. A. 107 (8), 3424–3429.

(32) Goosy, M., Harris, T. L., Tricoche, N., Javor, S., Lustgman, S., and Janda, K. D. (2015) Onchocerca volvulus Molting Inhibitors Identified through Scaffold Hopping. ACS Infect. Dis. 1 (5), 198–202.

(33) Pozarowski, P., and Darzyńskiwicz, Z. (2004) Analysis of cell cycle by flow cytometry. Methods Mol. Biol. 281, 301–311.

(34) Desai, P. V., Raub, T. J., and Blanco, M. J. (2012) How hydrogen bonds impact P-glycoprotein transport and permeability. Bioorg. Med. Chem. Lett. 22 (21), 6540–6548.

(35) Raub, T. J. (2006) P-glycoprotein recognition of substrates and circumvention through rational drug design. Mol. Pharmacol. 3 (1), 3–25.

(36) Fischer, C., Urriza, I. I., Bulman, C. A., Lim, K. C., Gut, J., Lachau-Durand, S., Engelen, M., Quirynen, L., Tekle, F., Baeten, B., Beernstsen, B., et al. (2019) Efficacy of subcutaneous doses and a new oral amorphous solid dispersion formulation of flubendazole on male jirds (Meriones unguiculatus) infected with the filarial nematode Brugia pahangi. PLoS Neglected Trop. Dis. 13 (1), No. e0006787.

https://dx.doi.org/10.1021/acsinfecdis.9b00397
ACS Infect. Dis. 2020, 6, 180–185