DNDI-6148: A Novel Benzoxaborole Preclinical Candidate for the Treatment of Visceral Leishmaniasis

Charles E. Mowbray,* Stéphanie Braillard, Paul A. Glossop, Gavin A. Whitlock, Robert T. Jacobs, Jason Speake, Bharathi Pandi, Bakela Nare, Louis Maes, Vanessa Yardley, Yvonne Freund, Richard J. Wall, Sandra Carvalho, Davide Bello, Magali Van den Kerkhof, Guy Caljon, Ian H. Gilbert, Victoriano Corpas-Lopez, Iva Lukac, Stephen Patterson, Fabio Zuccotto, and Susan Wyllie*

Cite This: J. Med. Chem. 2021, 64, 16159−16176

ABSTRACT: Visceral leishmaniasis (VL) is a parasitic disease endemic across multiple regions of the world and is fatal if untreated. Current therapies are unsuitable, and there is an urgent need for safe, short-course, and low-cost oral treatments to combat this neglected disease. The benzoxaborole chemotype has previously delivered clinical candidates for the treatment of other parasitic diseases. Here, we describe the development and optimization of this series, leading to the identification of compounds with potent in vitro and in vivo antileishmanial activity. The lead compound (DNDI-6148) combines impressive in vivo efficacy (>98% reduction in parasite burden) with pharmaceutical properties suitable for onward development and an acceptable safety profile. Detailed mode of action studies confirm that DNDI-6148 acts principally through the inhibition of Leishmania cleavage and polyadenylation specificity factor (CPSF3) endonuclease. As a result of these studies and its promising profile, DNDI-6148 has been declared a preclinical candidate for the treatment of VL.

INTRODUCTION

Visceral leishmaniasis (VL) is a poverty-linked disease, and occurs primarily in poor populations across Asia, East Africa, and South America. Current estimates suggest 50 000−90 000 new cases of VL occur each year. VL is caused by infection with the protozoan parasites Leishmania donovani and Leishmania infantum, with transmission mediated by the bite of the female phlebotomine sand fly. Once the human host is infected, parasites survive and multiply within macrophages, leading to symptoms including prolonged fever, enlarged spleen and liver, substantial weight loss, and progressive anemia. If left untreated, the vast majority of clinically symptomatic VL patients die within months.

The current standard treatment for patients with VL in East Africa is sodium stibogluconate (SSG) combined with paromomycin (PM). In South-East Asia, the standard treatment is liposomal amphotericin B (LAB), with paromomycin and miltefosine as a second-line treatment option. While these combination approaches are more effective than SSG monotherapy, the mainstay of VL treatment for many years, they still have significant limitations such as cost, route of administration (all given via the parenteral route apart from the oral drug miltefosine), and toxicity. Consequently, there is an ongoing need for effective new treatments that are easy to administer via oral dosing, are effective against VL in different regions of the world, have an improved safety profile, and are affordable for the patients who require them. Research and development for the treatment of VL has evolved rapidly in recent years, with 5 drug candidates from 4 different classes now in phase-1 clinical studies (Table 1).

Here, we outline the optimization of a benzoxaborole series toward a possible new therapy for VL. The benzoxaborole class of compounds has already successfully delivered a clinical
Clinical trials with DNDI-6148 are now underway.11 Coupling with intermediate acid intermediates R 2CO2H is described in detail in the Supplementary Information. Example Method A. Examples

\[ R_1\text{N} - \text{CO}_2\text{H} + \text{R}_2\text{CO}_2\text{H} \rightarrow R_1\text{N}-CO_2R_2 \]

was synthesized using Method A: HOBt, EDCI, DIPEA, tetrahydrofuran (THF), rt, 16 h, 4−68%. Zemplén−Houk coupling (THF), rt, 16 h, 4−68%. Choice was driven by substrate compatibility: Method A (HOBt/EDCI coupling), Method B (HATU coupling), and Method C (acid chloride formation using SOCl₂, followed by coupling with intermediate 2). The synthesis of the carboxylic acid intermediates R\(^{\text{II}}\)CO₂H is described in detail in the Supplementary Information. Example 12 was synthesized using Method A. Examples 4, 6−11, 13−15, 17−18, 20−21, 23−27, and 29−30 were synthesized using Method B, while examples 5, 16, 19, 22, 28, and 31 were synthesized using Method C.

Preclinical candidate compound 23 was synthesized using a 4-step synthesis (Scheme 2).

Formation of the fused tetrazole 23-2 was achieved by the reaction of 2-bromopyridine with sodium azide. Cyclo-addition of 23-2 with ethyl 3-oxobutanoate gave the N1-substituted triazole 23-3, which was hydrolyzed to the corresponding acid 23-4. Coupling benzoxaborole scaffold 2 using Method B gave the final compound 23 in high overall yield.

Compound 1 was potent in in vitro assays against Trypanosoma brucei, the etiological agent of HAT (EC\(_{50}\) value of 795 nM),12 but demonstrated only weak activity in intramacrophage assays with both L. infantum and L. donovani (Table 2). It should be noted that L. infantum-derived infections can be refractory to some current drug therapies. Thus, new compounds in development are screened against both L. infantum and L. donovani at an early stage to ensure efficacy. However, the close analogue 3 (SCYX-6759), lacking the gem-dimethyl substitution on the benzoxaborole ring, was considerably more potent in these assays.13

Previous studies demonstrated that compound 3 has low clearance in multiple animal models and is efficacious in a murine model of HAT.13 Consequently, compound 3 was progressed to an L. infantum hamster model of VL infection (Table 3).13 Compound 3 was highly efficacious at QD doses of 50 and 100 mg/kg, virtually clearing all parasites from the liver and spleen. However, reductions in parasite burden in the bone marrow were more modest and thus failed to meet the DNDI target candidate profile (TCP) of >95% reduction in parasite burden. Therefore, additional benzoxaboroles were synthesized to identify compounds that were highly efficacious in all tissues across a broad dose range.

The simplified benzoxaborole ring system, with the gem-dimethyl substitution on the benzoxaborole ring removed, was retained in subsequent analogues and the amide substituent became the focus for development. Replacing the lipophilic aryl substituent with a more polar heteroaryl group was investigated in detail to reduce the lipophilicity to values more associated with orally bioavailable drugs.13 Consistent with this design strategy, a range of substituted pyrazoles was synthesized (Table 4). Excellent levels of in vitro potency were achieved with compounds 4−6, which contain a trifluoromethyl R\(^{\text{I}}\) substituent and a fluorinated N-alkyl R\(^{\text{II}}\).
Table 2. Collated EC₅₀ Values for Compounds 1, 3 and the Current Front-Line Antileishmanial Miltefosine

| compound          | intramacrophage L. donovani EC₅₀, μM | intramacrophage L. infantum EC₅₀, μM | PMM    |
|-------------------|--------------------------------------|-------------------------------------|--------|
| 1                 | 59                                   | >64                                 | >64    |
| 3                 | 1.2                                  | 2.7                                 | >64    |
| 23                | 1.4                                  | 1.8                                 | >64    |
| miltefosine       | 10                                   | 10                                  | 33     |

*aGeometric mean value of at least three independent assays. PMM: primary mouse macrophages.*

Table 3. In Vivo Efficacy of Compound 3 in an L. infantum Hamster Model of VL, after QD Dosing for 5 Days

| dose (mg/kg) | reduction in parasite burden, % |
|--------------|---------------------------------|
|              | liver | spleen | bone marrow |
| 25           | 87.8  | 81.8   | 81.6        |
| 50           | 97.4  | 95.0   | 91.2        |
| 100          | 98.6  | 96.0   | 88.3        |

*n = 6 animals per group, results were expressed as a percentage reduction in amastigote burden compared to vehicle-treated, infected control animals.*

Typically, a HamLM clearance of <100 μL/min/mg is required to observe meaningful exposure in vivo. Further lipophilic aryl R² substituents such as 4-Cl-phenyl 13 and 2-MeO-phenyl 14 had potent in vitro L. infantum activity but were cytotoxic in PMM and/or MRC5 cells. The pyrazole 15 exhibited a significant drop in potency compared to its isomeric analogue 6, whereas the pyridyl-substituted compound 16 was slightly more potent than its isomer 11. Further substituted pyridine examples 17 and 18 were also potent and demonstrated good SI. In addition, compounds 16–18 were stable in both HLM and HamLM. Additional pyrazole isomers 19–21 all failed to combine sufficient in vitro potency and weak cytotoxicity with good metabolic stability.

Of the compounds made up to this point, 2-pyridyl-substituted analogue 16 was one of the most interesting as it combined good in vitro potency, low lipophilicity, promising SI, and was stable in both HLM and HamLM. For the next set of compounds, the 2-pyridyl substituent was retained and further changes to the 5-membered heterocycle were investigated (Table 5). Triazole 22, where R² = H, showed an encouraging improvement in both in vitro potency and HamLM stability over pyrazole 16. The Me-substituted example 23 had an even better profile with high levels of L. infantum potency, no sign of cytotoxicity in PMM and MRC5, and very high levels of metabolic stability in HLM and HamLM. Further 1,2,3-triazoles demonstrated that the 2-pyridyl substituent was important; for example, the phenyl example 24 and 3-pyridyl example 27 had significantly weaker in vitro potency. Substitution on the 2-pyridyl group could be tolerated, with the 6-OHMe analogue 25 showing an improved potency profile but at the expense of metabolic stability. The 6-Me compound 26 lost some potency and was therefore not progressed to metabolic stability assessment. Furan example 28 was very potent, and although it did exhibit some cytotoxicity in both PMM and MRC5, the high level of potency meant that 28 still retained a high SI. Despite being more lipophilic, metabolic stability was retained in both HLM and HamLM and, therefore, 28 was considered for further progression. The isomeric furan 29 had slightly reduced potency and SI compared to 28, and the thiophene 30 was highly cytotoxic in MRC5 cells. The more polar oxadiazole 31 delivered a good overall profile and was also considered for further progression.

substituent. Importantly, compounds 4–6 showed no evidence of cytotoxicity (CC₅₀ > 38 μM) in the counter-screen with PMM. These analogues also had excellent in vitro metabolic stability in HLM, but compounds 4 and 6 had poor stability in hamster liver microsomes (HamLM), which precluded their evaluation in the in vivo hamster model of VL. Compound 5 combined sufficient in vitro potency, selectivity index (SI), and stability in both HLM and HamLM to be considered for further progression. Compound 7 (R¹ = Me) was slightly less potent than its CF₃ analogue 6, and this same trend was also observed for the N-cyclobutyl examples 8 and 9 as well as for the 2-pyridyl examples 11 and 12. However, the CF₃-substituted analogues 6, 9, and 12 were all more cytotoxic in PMM than their CH₃-substituted counterparts 7, 8, and 11. Tetrahydroxynyl-substituted analogue 10 combined many of the necessary properties, but HamLM stability was insufficient to deliver enough in vivo exposure for efficacy studies.
The most promising compounds from this SAR investigation, namely 5, 16, 23, 28, and 31, were also assessed for their in vitro macrophage potency using intracellular *L. donovani* (Table 6). Pleasingly, four of these compounds retained excellent levels of *L. donovani* potency; however, the oxadiazole 31 was about 5-fold weaker against *L. donovani* compared with its *L. infantum* potency.

Compounds 5, 16, 23, 28, and 31 were also progressed to hamster oral pharmacokinetic (PK) studies and an *L. infantum* model of VL in hamsters (Table 7). Triazole 23 exhibited noticeably higher exposure after a single oral dose of 50 mg/kg.
compared with the other lead compounds, which is consistent with its higher in vitro stability in HamLM and indicated that in vitro metabolic clearance may be predictive of in vivo clearance for this set of compounds.

All five compounds demonstrated excellent efficacy, with parasitemia significantly reduced in all organs after a dose of 50 mg/kg BID for 5 days. Triazole 23 and furan 28 also maintained excellent efficacy at a lower dosing of 25 mg/kg BID, consistent with the combination of good potency and high exposure observed for 23 and the very high potency of 28. Extending the duration of treatment from 5 to 10 days for compound 23 led to almost complete eradication of parasites in the liver, spleen, and bone marrow. In contrast, the efficacy profile of compound 28 was not improved by extending the dosing period.

Compounds 23 and 28 were then studied in an L. donovani hamster model (Table 8). Triazole 23 exhibited high levels of efficacy in the liver and spleen when dosed orally at 50 mg/kg BID for 5 days, although the reduction in parasite load in bone marrow was lower than the target of 95%. However, by extending the dosing period to 10 days, very high efficacy across all three tissues was achieved at a dose of 25 mg/kg BID. In addition, furan 28 achieved excellent efficacy in all tissues at both 25 mg/kg BID and 50 mg/kg BID for 5 days. These important data demonstrated that for both L. infantum and L. donovani, potent in vitro and in vivo activity could be achieved.

Compounds 23 and 28 were also assessed in mouse models of L. donovani and L. infantum; data confirmed that the high levels of efficacy observed in the chronic hamster model of infection was also achieved in the acute mouse model (Table 9).

Based on their in vitro potency, metabolic stability, and in vivo pharmacokinetic and efficacy profiles, compounds 23 and 28 were investigated in more detail to determine if either could be progressed to further preclinical development.
by the outstanding in vivo efficacy of compound 28, a 14-day exploratory toxicity study was conducted in male and female Sprague-Dawley rats. Unfortunately, compound 28 caused significant multiorgan toxicity following daily dosing of 25 mg/kg and the NOAEL (No Observed Adverse Effect Level) was not determined (<12.5 mg/kg). Consequently, further development of compound 28 was halted.

One potential concern associated with compound 28 was the presence of the furan, a functional group that can undergo oxidative metabolism to reactive metabolites. Thus, 23 and 28 were investigated to determine potential routes of metabolism and to understand if protein—ligand adducts were formed when the compounds were incubated in microsomes in the presence of glutathione. Metabolite identification studies with compound 28 showed evidence of ring-opening of the furan to an enone M-12 metabolite in all species tested (rat, mouse, dog, human, hamster). This metabolite has the potential to undergo conjugation to form protein—ligand adducts (Scheme 3).

In contrast, incubation of triazole 23 with microsomes indicated very limited metabolism, with only small amounts of mono-oxidation and hydrolysis/oxidative deboronation observed in dog and human microsomes, respectively (Scheme 4). No metabolism was detected in rat microsomes, and no evidence of reactive metabolite formation was found in any species.

In addition to the safety signals raised by a moderate in vitro activity against the MRC5 cell line (Table 5) and the lack of efficacy improvement when treatment duration was extended in the hamster model (Table 7), these in vitro metabolic studies clearly identified a potential weakness with furan 28 that may contribute to the observed in vivo toxicity. Therefore, 23 was prioritized for further evaluation. CYP inhibition was weak (IC_{50} > 50 \mu M vs CYP1A2, 2C9, 2C19, 3A4, 2D6), hERG selectivity was high (hERG inhibition < 20% at 30 \mu M), and no activity > 50% was observed when 23 was tested in a panel of 88 targets (Cerep panel, Figure S13) at a concentration of 10 \mu M. Plasma-protein binding was moderate and consistent across species (dog 87%, human 92%, mouse 93%, rat 92%, hamster 88%). Thermodynamic solubility in physiologically relevant media was low to moderate (Fasted State Simulated Intestinal Fluid, FaSSIF solubility = 5.4 \mu g/mL, Fed State Simulated Intestinal Fluid, FeSSIF solubility = 6.3 \mu g/mL), and membrane permeability was good with no evidence of P-gp-mediated efflux (MCDK efflux ratio of 0.7, Table S3). Microsomal and hepatocyte stability was excellent across all species (Table 10), although hepatocyte stability was slightly worse in dogs compared with rats, monkeys, and humans. Compound 23 was also negative when tested in an Ames assay (TA98 and TA100 bacterial strains) with and without metabolic activation over a dose range from 1.5 to 1000 \mu g/well, suggesting no clastogenicity. To complete the early safety profile of compound 23, a 14-day exploratory toxicology study was conducted in male and female Sprague-Dawley rats where the no adverse effect limit was set at 50 mg/kg/day.

In vivo pharmacokinetics after i.v. and p.o. dosing was determined in rats and dogs (Table 11 and Figure S12). Clearance in rats was low (4.5 mL/min/kg) with comparatively higher clearance in dogs (13.7 mL/min/kg), in line with the higher turnover in dog hepatocytes compared with rat hepatocytes. Oral bioavailability in rats was very high, indicating complete oral absorption. Bioavailability was somewhat lower in dogs and not fully understood at this point, but still acceptable for future progression of the compound. Allometric scaling from available rat and dog PK data suggests that compound 23 will have low clearance (Cl = 1–4 mL/min/kg) and moderate–high bioavailability (40–90%) in humans. BID dosing in humans at 3–20 mg/kg is predicted to achieve the efficacious exposure observed in the L. infantum and L. donovani hamster and mouse models of VL.

Mode of Action Studies (MoA). MoA studies as an integrated part of a drug discovery program can provide vital information that can be used to combat the high failure rates associated with the development of phenotypically active compounds. The association of active compounds with defined molecular targets during the development process can be extremely powerful. Understanding compound MoA can allow toxic liabilities associated with the target to be directly assessed, prevent enrichment of drug candidates against the same molecular target, and halt the development of inhibitors with an unattractive or invalidated target. Furthermore, this knowledge can inform future drug combination strategies.

Our previous studies with acoziborole (ANS568, 1), in clinical development for HAT, revealed that this promising benzoxaborole specifically targets the Cleavage and Polyadenylation Specificity Factor 3 (CPSF3) in T. brucei. With additional compounds from within this series were also shown to target CPSF3, including 3. CPSF3 is an endonuclease that forms part of the CPSF complex, involved in the control of polyadenylation and trans-splicing of pre-mRNA. Indeed, CPSF3 orthologues have also been identified as the molecular targets of benzoxaboroles active against Plasmodium falciparum, Toxoplasma gondii, and Cryptosporidium spp. 21

With this in mind, we hypothesized that DNDJ-6148 (23) might
also target this important endonuclease in \textit{L. donovani}. To test this hypothesis, we first assessed the potency of DNDI-6148 (23) against \textit{T. brucei} bearing a mutation in the active site of CPSF3 (Asn$^{219}$His), previously demonstrated to confer resistance to acoziborole (1).\textsuperscript{19} Bloodstream trypanosomes bearing this specific mutation were 1.8-fold less sensitive to DNDI-6148 (23) compared to wild-type parasites (Figure 2A). Similarly, trypanosomes overexpressing the wild-type version of CPSF3 were 2.9-fold less sensitive to DNDI-6148 (23) (Figure 2B). Collectively, these data suggest that, like acoziborole (1), this benzoxaborole specifically targets CPSF3 in \textit{T. brucei}.

To determine if these boron-containing compounds specifically target CPSF3 in \textit{L. donovani}, transgenic promastigotes were generated overexpressing either CPSF3$^{\text{WT}}$ or CPSF3 bearing an Asn$^{219}$His mutation, equivalent to the Asn$^{232}$His mutation in the \textit{T. brucei} enzyme. Elevated levels of the wild-type and mutated CPSF3 in these cell lines were confirmed by label-free quantification (Figure S2). Overexpression of CPSF3$^{\text{WT}}$ in \textit{L. donovani} promastigotes did not substantially affect susceptibility to either acoziborole (1) (Figure 2C) or DNDI-6148 (23) (Figure 2D). However, promastigotes overexpressing the mutated version of this enzyme were significantly less sensitive to both compounds, demonstrating a 5-fold and 3.6-fold reduction in susceptibility to acoziborole (1) and DNDI-6148 (23), respectively (Figure 2C,D). The shift in potency observed with parasites overexpressing mutated CPSF3 was found to be specific for benzoxaboroles. Compound 3 elicited a similar shift in potency, while the established N-myristoyltransferase inhibitor DDD100097\textsuperscript{22} did not (Figure S3). The fact that this single Asn$^{219}$His mutation in the active site of CPSF3 has a marked effect on the potencies of both acoziborole (1) and DNDI-6148 (23) provides strong evidence that this endonuclease is the molecular target of this preclinical candidate.

**Precision Base Editing of \textit{LdCPSF3}.** We next utilized precision base editing with Cas9 to further probe the interactions between DNDI-6148 (23) and \textit{L. donovani} CPSF3. In the first instance, a template encoding a specific Asn$^{219}$His mutation was provided to repair a Cas9-induced lesion within CPSF3. Transfected parasites were then selected with DNDI-6148 (23) (6 $\mu$M). The resulting DNDI-6148-resistant population was clononed, genomic DNA was harvested, and the CPSF3 was sequenced to ensure that the desired edit had been successfully introduced. In all cases, these parasites maintained the Asn$^{219}$His encoded by the repair template and were consistently 3-fold less sensitive to DNDI-6148 (23) than wild-type (Table S2). As in our previous studies with \textit{T. brucei} CPSF3, attempts to edit Asn$^{219}$His to Tyr to mimic the human CPSF3 enzyme at this position were not tolerated by \textit{L. donovani}. However, using a degenerate repair template, parasites were recovered, maintaining Asn$^{219}$His and also Glu$^{226}$Val homoyzogous mutations. These doubly mutated parasites were >5-fold less sensitive to DNDI-6148 (23) (Table S2). Collectively, these data provide compelling evidence that DNDI-6148 specifically targets the endonuclease CPSF3 in \textit{L. donovani}. These data are also consistent with a recently published study reporting CPSF3 as amongst the top “hits” following the selection of the Cos-Seq genome-wide overexpression library with DNDI-6148.\textsuperscript{23}

**Molecular Modeling.** A homology model of the \textit{L. donovani} CPSF3 was generated using the crystallographic structure of the \textit{Thermus thermophilus} TTHA0252 homologue as a template (PDB code 3IEM—Figure 3). The \textit{Leishmania} enzyme shares 30% sequence identity with this bacterial homologue. The proposed binding mode for DNDI-6148 (23) in the model is consistent with the binding of the benzoxaborole acoziborole to the catalytic site of CPSF3 located at the interface of the metallo-$\beta$-lactamase and $\beta$-CASP domains in \textit{T. brucei} CPSF3.\textsuperscript{19} The site comprises two zinc atoms coordinated by a network of conserved histidine and aspartic acid residues. Interaction with a zinc-activated water molecule leads to the formation of a negatively charged tetrahedral boronate species that coordinates the zinc atom (Figure S4), mimicking the transition state of the phosphate of the RNA substrate. The amide in position 6 of the benzoxaborole moiety directs the pyridyl-triazole moiety of

---

**Table 10. In Vitro Metabolic Stability Across Species for Compound 23**

| species     | mouse Cl$_{\text{uu}}$ ($\mu$L/min/mg protein) | rat Cl$_{\text{uu}}$ ($\mu$L/min/mg protein) | dog Cl$_{\text{uu}}$ ($\mu$L/min/mg protein) | human Cl$_{\text{uu}}$ ($\mu$L/min/mg protein) |
|-------------|-----------------------------------------------|-------------------------------------------|------------------------------------------|------------------------------------------|
| microsome   | <11.9                                         | <11.9                                     | 5.8                                      | <11.9                                     |
| hepatocyte  | n.d.                                          | 0.7                                       | 2.4                                      | 0.5                                       |

$n$ = 3 male SD rats, 2 mg/kg i.v., 10 mg/kg p.o. $^b n$ = 2 male Beagle dogs, 1 mg/kg i.v., 5 mg/kg p.o.

**Table 11. In Vivo Pharmacokinetics of 23 in Rat and Dog**

| species | rat$^a$ | dog$^b$ |
|---------|---------|---------|
| route of administration | i.v. | p.o. | i.v. | p.o. |
| clearance (mL/min/kg) | 4.5 | 13.7 | 4.5 | 13.7 |
| $V_d$ (L/kg) | 0.6 | 1.3 | 0.6 | 1.3 |
| $T_{1/2}$ (h) | 2.8 | 1.5 | 2.8 | 1.5 |
| $F$ (%) | 94 | 39 | 94 | 39 |

$^a n$ = 3 male SD rats, 2 mg/kg i.v., 10 mg/kg p.o. $^b n$ = 2 male Beagle dogs, 1 mg/kg i.v., 5 mg/kg p.o.
DNDI-6148 (23) toward the area occupied by the terminal uracil base of the RNA substrate and establishes a π-stacking interaction with Tyr270 (Figure 3A). Additionally, the amide NH forms a hydrogen bond with the hydroxyl group in the Thr218 side-chain. There seems to be no direct interaction between DNDI-6148 (23) and the Asn219 residue, where mutation to His is associated with resistance (Figure 3B). However, we propose that mutation to a bulkier His residue in this position has a negative impact on DNDI-6148 (23) binding due to steric clashes with the methyl pyridyl-triazole moiety of the compound (Figure 3C). This likely prompts the ligand to adopt a different binding mode where a hydrogen bond with Thr218 is lost, the hydrophobic methyl is directed toward the solvent, and the overall ligand conformation is strained. Undoubtedly, there is a high degree of similarity surrounding the proposed binding site of DNDI-6148 in the parasite enzyme and the human homologue, with 21 identical residues out of the 26 within 5 Å from the bound ligand. However, Asn219 in the parasite enzyme is replaced by a tyrosine residue in the human homologue. This bulkier tyrosine residue is likely to cause severe steric hindrance that prevents DNDI-6148 from binding to the human and is entirely consistent with the favorable selective toxicity profile of this compound.

The second mutation (Glu229Val) is at the beginning of helix 7 in the β-CASP domain of CPSF3. Helix 7 is some way from the catalytic site but is connected to the loop that contains Thr218, Asn219, and Ile221, defining the pocket that recognizes the pyridine ring of DNDI-6148 (23). In our models, a mutation from Glu229 to Val would result in a structural rearrangement of the loop, changing the morphology of the binding site and ultimately impacting the ligand recognition event.

**CONCLUSIONS**

We have optimized a series of benzoxaboroles and identified a range of compounds with very potent *in vitro* antileishmanial activity against *L. infantum* and *L. donovani*, starting from benzamide-substituted compound 3. Variation of the amide substituent was explored extensively, and a range of heterocyclic amide groups was found to deliver potent *in vitro* activity combined with good metabolic stability. Several compounds were assessed in the *L. infantum* hamster model of VL, with compound 23 standing out by exhibiting very high levels of efficacy in all organs at doses of 25 mg/kg BID for 5 or 10 days. Additional *in vivo* studies in the *L. donovani* hamster model also indicated high levels of efficacy, demonstrating that 23 was equally efficacious against both *Leishmania* species responsible for causing VL. Efficacy of 23 was also confirmed in *L. donovani* and *L. infantum* BALB/c mouse models. Further study of compound 23 indicated that it had excellent pharmacokinetics, a good *in vitro* safety profile, and met all of the criteria in the DNDI/TCP for VL. Comprehensive MoA studies confirm that DNDI-6148 (23) targets the endonuclease CPSF3, a previously unexploited drug target in *Leishmania* spp. Consequently, 23 (DNDI-6148) was...
nominated as a preclinical candidate and further development toward clinical studies is ongoing. Furthermore, compound 23 demonstrates similar levels of in vitro and in vivo activity against species of Leishmania responsible for causing cutaneous leishmaniasis (CL), supporting the development of this candidate for multiple forms of leishmaniasis. It should be noted that we have also generated data that supports the development of DNDI-6148 for Chagas’ disease. This information will be disclosed in due course. In conclusion, the data package for DNDI-6148 (23) provides every reason to believe that this benzoxaborole can become a much-needed safe oral treatment for patients suffering from this devastating neglected tropical disease.

**EXPERIMENTAL SECTION**

**Chemistry. Purity.** All compounds reported in this study are >95% pure as determined by high-performance liquid chromatography (HPLC) analysis. HPLC chromatograms of a representative compound (3) and all analogues used in in vivo experiments (5, 16, 23, 28, 31) are shown in the Supplementary Information (Figures S6–S11).

**General.** Unless otherwise indicated, all reactions were magnetically stirred under an inert atmosphere. All reagents, including solvents, were used as received. Anhydrous solvents were dried in-house by passing through activated alumina. Thin-layer chromatography was performed on glass-backed precoated silica gel 60 plates, and compounds were visualized using UV light or iodine. Silica gel column chromatography was performed using 200–300-mesh silica gel. Preparative HPLC was performed using Phenomenex Synergi C18 150 mm × 30 mm, 4 μm, (2) YMC-pack ODS-AQ 150 mm × 30 mm, 5 μm, (3) Agela Venusil ASB C18 150 mm × 21.2 mm, 5 μm, or (4) Boston Symmetry C18 ODS-R 150 mm × 30 mm, 5 μm; elution was performed with 0.225% (by volume) of formic acid in water (solvent A) and acetonitrile (solvent B); fractions containing products were lyophilized. NMR spectra were recorded on a Bruker AVANCE 400 MHz spectrometer in the solvents specified. Liquid chromatography–mass spectrometry (LC-MS) spectra were recorded on an Agilent 1200 or Shimadzu 2020 spectrometer equipped with electrospray ionization, quadrupole MS detector, and Chromolith Flash RP-18e 25 mm × 2.0 mm column, eluting with 0.0375% (by volume) of trifluoroacetic acid (TFA) in water (solvent A) and 0.01875% (by volume) of TFA in acetonitrile (solvent B). Analytical HPLC was performed using a Shimadzu LC20AB machine and one of two columns, chosen from (1) Ximate C18 2.1 mm × 30 mm, 3 μm or (2) CHROM-MATRIX Innovation C18 2.1 mm × 30 mm, 2.6 μm; elution was performed with 0.0375% (by volume) of TFA in water (solvent A) and 0.01875% (by volume) of TFA in acetonitrile (solvent B). The purity of final compounds was ≥96%, as determined by HPLC. Unless otherwise stated, final compounds were isolated as amorphous solids without collection of melting point data.

**General Procedures for the Preparation of Amides. General Procedure A.** To a solution of the corresponding acid (14.4 mmol, 1.0 equiv), HOBt (17.3 mmol, 1.2 equiv), EDCI (17.3 mmol, 1.2 equiv), and DIPEA (36.1, 2.5 equiv) in dichloromethane (DCM) (30 mL) was added 6-aminobenzoc[1,2]-oxaborol-1(3H)-ol (14.4 mmol, 1.0 equiv). The reaction mixture was stirred at room temperature for 2 h. Water (100 mL) was added, and the reaction mixture was extracted with DCM (100 mL × 3). The combined organic extracts were washed with brine (100 mL × 2), dried with anhydrous Na2SO4, filtered, and concentrated in a vacuum to give the crude product, which was purified by silica gel chromatography or preparative HPLC.

**General Procedure B.** To a solution of the corresponding acid (1.5 mmol, 1.0 equiv), HATU (1.9 mmol, 1.3 equiv), and DIPEA (2.8 mmol, 1.8 equiv) in DMF (3 mL) was added 6-aminobenzoc[1,2]-oxaborol-1(3H)-ol (1.5 mmol, 1.0 equiv). The mixture was stirred at room temperature overnight. The mixture was purified by preparative HPLC.

**Figure 3.** Molecular docking of DNDI-6148 into a model of the L. donovani CPSF3 active site. (A) Docking model for DNDI-6148 (23) (yellow structure) bound to L. donovani CPSF3. Gray spheres represent zinc atoms, blue dotted lines indicate a π stacking interaction, and the purple dotted line indicates a hydrogen bond interaction. (B) Close-up of key interactions involved in DNDI-6148 (23) (yellow structure) binding in the active site of CPSF3. (C) Modified docking model illustrating a steric clash between DNDI-6148 (23) (red patch) and the His residue (yellow) in Asn219His substituted CPSF3. The wild-type CPSF3 structure is shown in green.
**General Procedure C** A solution of the corresponding acid (10.5 mmol) in SOCl₂ (40 mL) was stirred at 50 °C for 4 h. The mixture was concentrated in a vacuum to give acyl chloride, which was used directly in the next step. To a solution of 6-amino[6,12]-oxaborol-3(3H)-ol (1.2 g, 8.1 mmol) and DIPEA (2.9 mL, 16.7 mmol) in THF (30 mL) was added the acid chloride (8.1 mmol). The mixture was stirred at room temperature for 16 h. Water (100 mL) was added, and the reaction mixture was extracted with EtOAc (100 mL × 3). The combined organic extracts were washed with brine (100 mL × 2), dried with anhydrous Na₂SO₄, filtered, and concentrated in a vacuum to give the crude product, which was purified by silica gel chromatography or preparative HPLC.

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-1,3-bis(trifluoromethyl)-1H-pyrazole-4-carboxamide (4)** The title compound was synthesized according to method B using a mixture of 1,3-bis(trifluoromethyl)-1H-pyrazole-4-carboxylic acid and 1,3-bis(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (4 g, 16 mmol), HATU (7.3 g, 19.2 mmol), 6-aminobenzo[c][1,2]oxaborol-3(3H)-ol 2 (2.9 g, 19.2 mmol), DIPEA (4.1 g, 32 mmol), and DMF (30 mL) to yield 6.5 g of crude product as a yellow solid. 2 g of the crude product was purified by prep-HPLC to yield 4 as a white solid (200 mg). HPLC: 100% pure. MS (ESI) m/z = 380.1 [M + 1]⁺. ¹H NMR (400 MHz, DMSO-d₆): δ = 10.54 (s, 1H), 9.44 (s, 1H), 9.32 (brs, 1H), 8.18 (s, 1H), 7.65 (d, J = 7.2 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 4.98 (s, 2H). HRMS (ES+): calcd for C₁₃H₁₁B₁F₋N₃O₃ [M+H]+ 380.0641, found 380.0644 (0.75 ppm).

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-1-(2,2,2-trifluoroethyl)-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (5)** To a cold solution of 6-amino-3,3-dimethylbenzo[c][1,2]oxaborol-1(3H)-ol (32.7 g, 152.3 mmol) and DIPEA (39.3 g, 305.2 mmol) in THF (500 mL) was added a solution of 1-(2,2,2-trifluoroethyl)-1H-pyrazole-4-carboxyl chloride (42.7 g, 152.5 mmol) in THF (150 mL) dropwise. The mixture was stirred at room temperature for 4 h and concentrated in a vacuum to a residue that was recrystallized from MeCN and water to yield 5 as a gray solid (41.1 g, 68%). HPLC: 98% pure. MS (ESI) m/z = 394.2 [M+H]⁺. ¹H NMR (400 MHz, DMSO-d₆): δ = 10.37 (s, 1H), 9.27 (s, 1H), 8.72 (s, 1H), 8.16 (s, 1H), 7.66 (dd, J = 2.0, 8.0 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 5.48 (q, J = 9.2 Hz, 2H), 4.97 (s, 2H).

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (6)** The title compound was synthesized according to method B using 3-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid 6 (18.0 g, 65.2 mmol), HATU (34.7 g, 91.3 mmol), 1-hydroxy-3H-2-benzoxaborol-6-amine 2 (10.7 g, 71.8 mmol), DIPEA (16.9 g, 131.0 mmol), and DMF (150 mL) to give a residue. The residue was purified by silica gel chromatography (elution with ethyl acetate/petroleum ether/AcOH = 1: 3: 0.1) to give the crude product, which was recrystallized from MeOH to yield 6 as a white solid (9.60 g, 36.2%). HPLC: 99% pure. MS (ESI) m/z = 408.1 [M + 1]⁺. ¹H NMR (400 MHz, DMSO-d₆): δ = 10.22 (s, 1H), 9.25 (s, 1H), 8.66 (s, 1H), 8.17 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 4.96 (s, 2H), 4.56 (t, J = 6.8 Hz, 2H), 3.02 (m, 2H). HRMS (ES+): calcd for C₁₃H₁₁B₁F₄N₃O₃ [M+H]+ 408.0594, found 408.0597 (0.70 ppm).

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(3-methyl(trifluoromethyl)-1H-pyrazole-4-carboxamide (7)** The title compound was synthesized according to method B using a mixture of 3-methyl-1-(3,3,3-trifluoropropyl)-1H-pyrazole-4-carboxylic acid and 3-methyl-1-(3,3,3-trifluoropropyl)-1H-pyrazole-4-carboxylic acid (469 mg, 2.1 mmol), HATU (390 mg, 2.3 mmol), 6-aminobenzo[c][1,2]-oxaborol-3(3H)-ol 2 (300 mg, 2.0 mmol), HATU (390 mg, 2.3 mmol), DIPEA (520 mg, 4.0 mmol), and DMF (5 mL) to yield the crude product, which was purified by preparative HPLC to give 7 (207 mg, 29%) as a white solid and 15 (205 mg, 29%) as a white solid.

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-5-methyl-1-(3,3,3-trifluoropropyl)-1H-pyrazole-4-carboxamide (15)** The title compound was synthesized according to method B using a mixture of 3-methyl-1-(3,3,3-trifluoropropyl)-1H-pyrazole-4-carboxylic acid and 5-methyl-1-(3,3,3-trifluoropropyl)-1H-pyrazole-4-carboxylic acid (469 mg, 2.1 mmol), HATU (390 mg, 2.3 mmol), 6-aminobenzo[c][1,2]-oxaborol-3(3H)-ol 2 (300 mg, 2.0 mmol), HATU (390 mg, 2.3 mmol), DIPEA (520 mg, 4.0 mmol), and DMF (5 mL) to yield the crude product, which was purified by preparative HPLC to give 15 (207 mg, 29%) as a white solid.
The title compound was synthesized according to method B using 1-(3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl)carbamide (15.0 g, 73.9 mmol), HATU (722 mg, 1.9 mmol), 6-aminobenzo[c]1,2-oxaborol-1(3H)-ol (2.9 g, 19.4 mmol), and DMF (3 mL) to yield the crude product, which was purified by preparative HPLC to yield 13 as a white solid (180.0 mg, 62%). HPLC: 96% pure. MS (ESI) m/z = 321.1 [M+H]+. 1H NMR (400 MHz, DMSO-d6): δ = 8.65 (d, J = 4.4 Hz, 1H), 8.23 (s, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.87 (m, 2H), 7.78 (m, 2H), 7.41 (d, J = 8.0 Hz, 1H), 7.37 (m, 1H), 4.98 (s, 2H).

N-((1-(6-Chlorophenyl)-3-(tri-fluormethyl)-1H-pyrazole-5-carboxamido)-1H-pyrazole-4-carboxylic acid (20.0 mg, 0.8 mmol), DIPEA (0.2 mL, 1.4 mmol), and DMF (5 mL) were added to a solution of 3-(pyridin-2-yl)-1H-pyrazole-4-carboxamide (19 mg, 0.1 mmol), HATU (491 mg, 1.29 mmol), and DMF (3 mL) to yield the crude product, which was triturated with DCM. The precipitated product was collected by filtration and recrystallized from CHCl3 to give 14 (190 mg, 41%) as a white solid. 1H NMR (400 MHz, DMSO-d6): δ = 8.03 (d, J = 8.0 Hz, 1H), 7.97 (m, 2H), 7.71 (m, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.68 (m, 1H), 4.91 (s, 1H, ArH), 9.23 (s, 1H, OH), 8.88 (s, 1H, ArH), 7.32 (s, 1H, ArH), 9.89 (s, 1H, NH).

N-(1-Hydroxy-1,3-dihydro-2,1-benzoxaborol-6-yl)-1-methyl-3-1H-pyrazole-4-carboxamide (14). The title compound was synthesized according to method B using 1-(2-methoxypyridin-2-yl)-1H-pyrazole-4-carboxamide (180.0 mg, 0.62 mmol), HATU (210 mg, 0.6 mmol), DIPEA (89 mg, 0.57 mmol), and DMF (3 mL) to yield the crude product, which was purified by preparative HPLC to yield 18 as a white solid (34.8 mg, 22%). HPLC: 96% pure. MS (ESI) m/z = 349.1 [M+1]+. 1H NMR (400 MHz, DMSO-d6): δ = 7.95 (s, 1H), 8.23 (s, 1H), 8.15 (s, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 10.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.35 (m, 2H), 4.97 (s, 2H), 2.84 (s, 3H), 2.54 (s, 3H).

N-(1-Hydroxy-1,3-dihydrobenzimidazol-2(1H)-yl)-1H-pyrazole-5-carboxamide (19). To a solution of 6-amino[12]1,2-oxaborol-3(3H)-ol (357.0 mg, 2.4 mmol) and DIPEA (619.0 mg, 4.8 mmol) in MeCN (10 mL) was added a solution of 3-(pyridin-2-yl)-1H-pyrazole-5-carboxyl chloride (500 mg, 2.4 mmol) in MeCN (10 mL) dropwise. The mixture was stirred at 60 °C for 20 h. The mixture was concentrated in a vacuum to give a crude product, which was purified by preparative HPLC to yield 19 as a white solid (28.4 mg, 3.7%). HPLC: 96% pure. MS (ESI) m/z = 321.1 [M+1]⁺. 1H NMR (400 MHz, DMSO-d6): δ = 8.65 (d, J = 4.4 Hz, 1H), 8.23 (s, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 2.0, 8.0 Hz, 1H), 7.51 (s, 1H), 7.38 (m, 2H), 4.98 (s, 2H).

N-(1-Hydroxy-1,3-dihydrobenzimidazol-2(1H)-yl)-1-methyl-3-1H-pyrazole-4-carboxamide (20) and N-(1-Hydroxy-1,3-dihydrobenzimidazol-2(1H)-yl)-1-methyl-5-(pyridin-2-yl)-1H-pyrazole-3-carboxamide (21). The title compounds were synthesized according to method B using a mixture of 1-methyl-3-(pyridin-2-yl)-1H-pyrazole-5-carboxylic acid and 1-methyl-5-(pyridin-2-yl)-1H-pyrazole-3-carboxylic acid (300 mg, 1.5 mmol), HATU (722 mg, 1.9 mmol), 6-amino[12]1,2-oxaborol-3(3H)-ol (222.0 mg, 1.5 mmol), DIPEA (361.0 mg, 2.8 mmol), and DMF (3 mL) to yield the crude product, which was purified by preparative HPLC to give 20 (123 mg, 25%) as a white solid and 21 (407.0 mg, 8%) as a white solid.

N-(1-Hydroxy-1,3-dihydrobenzimidazol-2(1H)-yl)-1H-pyrazole-4-carboxamide (22). A solution of 6-aminobenzimidazol[1,2]1,2-oxaborol-1(3H)-ol (82.0 mg, 0.5 mmol), 6-amino[12]1,2-oxaborol-3(3H)-ol (282.0 mg, 0.5 mmol), HATU (210 mg, 0.6 mmol), DIPEA (89 mg, 0.57 mmol), and DMF (3 mL) to yield the crude product, which was purified by preparative HPLC to yield 23 as a yellow solid (4.0 mg, 74%). HPLC: 100% pure. MS (ESI) m/z = 336.1 [M+1]⁺. 1H NMR (400 MHz, DMSO-d6): δ = 10.59 (s, 1H), 9.25 (s, 1H), 8.70 (d, J = 4.8, 0.8 Hz, 1H), 8.28 (s, 1H), 8.19 (s, J = 8.0 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.72 (m, 1H), 7.41 (d, J = 8.4 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 2.81 (s, 3H), 2.63 (s, 3H), 2.21 (s, 3H), 2.14 (s, 3H).
7.83 (dd, J = 8.0, 2.0 Hz, 1H), 7.67 (d, J = 5.2 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 4.97 (s, 2H), 2.81 (s, 3H).

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (24).** The title compound was synthesized according to method B using 5-methyl-1H-1,2,3-triazole-4-carboxylic acid (870 mg, 5.5 mmol), 6-amino-benzo[c][1,2]oxaborol-1(3H)-ol (2) (80.0 mg, 0.5 mmol), HATU (110.0 mg, 0.50 mmol), DIPEA (6.8 g, 52.7 mmol) and a solution of 1-hydroxy-3-methyl-4-(pyridin-2-yl)furan-2-carboxylic acid (1.7 g, 8.3 mmol) in MeCN (50 mL) at 0 °C for 1 h. The reaction mixture was filtered and the cake was collected and purified by preparative HPLC to yield 27 as a white solid (45 mg, 35%). HPLC: 95% pure. MS (ESI): m/z = 335.0 [M + 1]+. 1H NMR (400 MHz, DMSO-d6): δ 10.16 (s, 1H), 9.24 (s, 1H), 8.28 (s, 1H), 8.07 (s, J = 8.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 4.98 (s, 2H), 2.82 (s, 3H), 2.59 (s, 3H). HRMS (ES+): calc. for C17H16B1N4O3 [M+H] + 335.1315, found 335.1313 (0.9 ppm).

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-1-(6-methylpyridin-2-yl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (25).** The title compound was synthesized according to method B using 5-methyl-1-(6-methoxypyridin-2-yl)-5-methyl-1H-1,2,3-triazole-4-carboxylic acid (870 mg, 5.8 mmol), 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol (2) (80.0 mg, 0.5 mmol), HATU (16.0 g, 42.1 mmol) in DMF (60 mL) was added 6-amino-benzo[c][1,2]oxaborol-1(3H)-ol (2) (4.2 g, 28.2 mmol). The mixture was stirred at room temperature overnight. Water (200 mL) was added and a lot of solid formed. The suspension was filtered and the cake was collected and purified by preparative HPLC to yield 29 as a white solid (6.5 g, 65.6%). HPLC: 100% pure. MS (ESI): m/z = 334.9 [M + 1]+. 1H NMR (400 MHz, DMSO-d6): δ 10.20 (s, 1H), 9.27 (s, 1H), 8.65 (d, J = 4.0 Hz, 1H), 8.17 (s, 1H), 7.88 (2H), 7.75 (m, 2H), 7.40 (d, J = 8.4 Hz, 1H), 7.30 (m, 1H), 4.98 (s, 2H), 2.76 (s, 3H). HRMS (ES+): calc. for C17H15B1N5O3 [M+H]+ 335.1305, found 335.1306 (0.6 ppm).

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-methyl-5-(pyridin-2-yl)thiophene-2-carboxamide (30).** The title compound was synthesized according to method B using 3-methyl-4-(pyridin-2-yl)thiophene-2-carboxylic acid (100 mg, 0.46 mmol), 6-amino-benzo[c][1,2]oxaborol-1(3H)-ol (2) (80 mg, 0.54 mmol), HATU (200 mg, 0.53 mmol), DIPEA (300 mg, 2.33 mmol), and DMF (2 mL) to yield the crude product, which was purified by preparative HPLC to yield 30 as a white solid (63.8 mg). HPLC: 99% pure. MS (ESI): m/z = 351.1 [M + 1]+. 1H NMR (400 MHz, DMSO-d6): δ 10.26 (s, 1H), 9.27 (s, 1H), 8.70 (d, J = 4.0 Hz, 1H), 8.11 (s, 1H), 8.03 (s, 1H), 7.74 (t, J = 2.0 Hz, 1H), 7.70 (m, 2H), 7.40 (m, 2H), 4.98 (s, 2H), 2.55 (s, 3H). HRMS (ES+): calc. for C18H15B1N4O2S [M+H]+ 351.0975, found 351.0978 (0.94 ppm).

**N-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-5-(pyridin-2-yl)-1,3,4-oxadiazole-2-carboxamide (31).** To a reaction mixture of 5-(pyridin-2-yl)-1,3,4-oxadiazole-2-carboxylic acid were added DIPEA (6.8 g, 52.7 mmol) and a solution of 1-hydroxy-3H-2,1-benzoxaborol-6-amine 2 (2.5 g, 17 mmol) in MeCN (50 mL) at 0 °C. The mixture was stirred at 0 °C for 2H. The reaction mixture was quenched by adding water (100 mL) at room temperature and extracted with EtOAc (100 mL × 2). The combined organic extracts were washed with brine (200 mL × 2), dried over Na2SO4 filtered, and concentrated under reduced pressure to give a residue. The residue was dispersed in DCM (20 mL), filtered, and the cake was dried under reduced pressure to give a crude product, which was purified by preparative HPLC to yield 31 as a white solid (1.9 g, 45%). HPLC: 99% pure. MS (ESI): m/z = 323.2 [M+H]+. 1H NMR (400 MHz, DMSO-d6): δ 11.36 (s, 1H), 9.32 (s, 1H), 8.84 (d, J = 2.4 Hz, 1H), 8.32 (m, 1H), 8.25 (s, 1H), 8.12 (1H), 7.85 (m, 1H), 7.72 (1H), 7.72 (d, J = 3.6 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 5.00 (s, 2H).

**Biology.** Compounds, and Reagents. For in vitro assays, compound stock solutions were prepared in 100% dimethyl sulfoxide (DMSO) at 20 mM. Compounds were serially pre-diluted (2-fold or 4-fold) in DMSO, followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of <1%. For in vivo studies, compounds were formulated in 2% ethanol, 1 mol equiv NaOH, and dextrose (5% solution) when administrated orally to mice, hamsters, and dogs or intravenously (pH adjusted to 7) to rats and dogs. For oral administration in rats, 0.5% (w/v) methylcellulose and 1% (w/v) sodium dodecyl sulfate in Mill-Q water was used. Mifelefosine was formulated in water at 20 mg/mL. **Cell Cultures.** Primary peritoneal mouse macrophages (PMM) were collected two days after peritoneal stimulation with a 2% peritoneal suspension. MRC5c197 cells (diploid human embryonic lung fibroblasts) were cultured in minimal essential medium (MEM) containing Earle’s salts, supplemented with 1-glutamine, NaHCO3, and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO2.
*Parasites. L. infantum* (MHOM/MA/67/ITMAP263) and *L. donovani* (MHOM/ET/67/L82) were maintained in the golden Syrian hamster (*Mesocricetus auratus*). *Ex vivo* amastigotes were collected from the spleen of an infected donor hamster using two centrifugation purification steps: 230g for 10 min, keeping the supernatant layer, and 4100g for 30 min, keeping the pellet. The spleen parasite burden was assessed using the Stauber technique. 

For in vitro assays, the inoculum was prepared in RPMI-1640 medium, supplemented with 200 mM l-glutamine, 16.5 mM NaHCO₃, and 5% inactivated fetal calf serum. For the *in vivo* model, an infection inoculum containing 2 × 10⁷ amastigotes/100 µL was prepared in phosphate-buffered saline (PBS).

**Animals.** Female golden hamsters for the *in vivo* model of visceral leishmaniasis were purchased from Janvier, France (body weight 80–100 g). This study using laboratory rodents was carried out in strict accordance with all mandatory guidelines (EU directives, including the Revised Directive 2010/63/EU on the protection of Animals used for Scientific Purposes that came into force on 01/01/2013, and the declaration of Helsinki in its latest version) and was approved by the ethical committee of the University of Antwerp, Belgium (UA-ECD 2011-74). Female golden hamsters for the pharmacokinetic study were purchased from Vital River, Beijing, China. This study was conducted following institutional review and in accordance with institutional and national guidelines at WuXi AppTec (the Institutional Animal Care and Use Committee (IACUC)). Balb/c mice for the *in vivo* model of visceral leishmaniasis (LSHTM) were purchased from Charles River, U.K., and related studies were conducted under license PPL 70/8427 from the U.K. Home Office.

**Intramacrophage L. infantum and L. donovani Assays.** The assay was performed in sterile 96-well microtiter plates, each well containing 10 µL of the compound dilution and 190 µL of the PMM/amastigote inoculum (3 × 10⁴ cells/4.5 × 10³ parasites per well). Parasite multiplication was compared to untreated infected controls (100% growth) and uninfected controls (0% growth). After five-day incubation, total parasite burdens were microscopically assessed after staining the cells with a 10% Giemsa solution. The results were expressed as a concentration reduction in parasite burden compared to untreated control wells. EC₅₀ values were determined using an extended dose range (2-fold compound dilutions, 8-point concentration curve). Mifepristone was used as the reference drug. For selected compounds, this assay format was also run using the *L. donovani* inoculum.

**In Vitro MRC5 and PMM Cytotoxicity Assays.** Assays were performed in sterile 96-well microtiter plates, each well containing 10 µL of the compound dilution and 190 µL of MRC5 or PMM inoculum (3 × 10⁴ cells/mL). Cell growth was compared to untreated controls (100% growth) and assay-media controls (0% growth). After three-day incubation, cell viability was assessed fluorometrically by adding resazurin (50 µL/well of a stock solution in phosphate buffer (50 µg/mL)), incubating for 4 h and measuring fluorescence (λₑₒ = 550 nm, λₘₐₓ = 590 nm). The results were expressed as a percentage reduction in cell growth compared to untreated control wells. EC₅₀ values were determined using an extended dose range (2-fold compound dilutions, 8-point concentration curve) to a highest concentration of 64 µM. Tamoxifen was included as the reference drug.

**In Vivo Hamster Model of Visceral Leishmaniasis.** Female golden hamsters were randomly allocated to experimental groups of six animals each, based on body weight. At the start of the experiment (Day 0), each animal was infected with *L. infantum* or *L. donovani* inoculum, delivered by intracardial injection. Six hamsters were assigned to the vehicle-treated, infected control group. Six hamsters were assigned per group (1 group/compound) for the evaluation of compound and mifepristone. At Day 21 postinfection (21 dpi), all animals in each group were dosed orally for five (or ten) consecutive days (21–25 dpi): compound was dosed at 25–50 mg/kg b.i.d.; mifepristone was dosed at 40 mg/kg q.d. At Day 35 (10 days after the final oral dose), all animals were euthanized and autopsies were conducted. The study evaluated the following parameters.

1. **Adverse effects:** all animals were observed daily for the occurrence/presence of adverse effects.
2. **Body weight:** all animals were weighed twice per week to monitor general health.
3. **Parasite burden:** amastigote burdens in each target organ (liver, spleen, and bone marrow) were determined at Day 35. The organs of individual animals were weighed (except for bone marrow). Impression smears were stained with Giemsa for microscopic examination of the total amastigote burden, defined as the mean number of amastigotes per cell multiplied by the number of cells counted (minimum 500 nuclei); the results were expressed as a percentage reduction in amastigote burden compared to vehicle-treated, infected control animals.

**In Vivo Mouse Model of Visceral Leishmaniasis.** Female BALB/c mice were infected with 2 × 10⁷ *L. donovani* amastigotes/0.2 mL L. harvested from the spleen of an infected donor RAG1B6 mouse (LSHTM). After 7 days, the mice were treated with mifepristone or DNDI-6148 for 5 consecutive days. Five days after the final dose, mice were humanely killed, liver and spleens were dissected and weighed, and impression smears were made for the calculation of parasite burden (Stauber equation). 

**Liver Microsome Stability.** Tests were performed by WuXi AppTec, China. Test compounds (at 1 µM) or positive controls (testosterone, propafenone, and diclofenac) were incubated at 37 °C with liver microsomes from Balb/c mouse, golden (Syrian) hamster, Sprague-Dawley rat, Beagle dog, or human in the presence of a NADPH regenerating system and phosphate buffer (100 mM, pH 7.4) at 0.4 or 0.8 mg/mL microsomal protein. The samples were removed at time intervals from 0 to 60, 90, or 120 min and immediately mixed with cold methanol supplemented or not with acid (3% formic acid) and centrifuged prior to analysis by LC-MS/MS using tolbutamide or propranolol as internal standards.

**Hepatocyte Stability.** Tests were performed by WuXi AppTec, China. Following a viability check of cryopreserved hepatocytes from Sprague-Dawley rat, Beagle dog, or human, test compounds (at 1 µM) or positive controls 7-ethoxycoumarin and 7-hydroxycoumarin (at 3 µM) were incubated at 37 °C with liver microsomes from Balb/c mouse, golden (Syrian) hamster, Sprague-Dawley rat, Beagle dog, or human in the presence of Williams’ Medium E. Following incubation of 15, 30, 60, 90, 120, 180, and 240 min, reactions were stopped by the addition of acetonitrile, the samples were centrifuged, and LC-MS/MS analysis was conducted using tolbutamide as an internal standard.

**Metabolite ID Studies.** Metabolite identification studies were conducted at WuXi AppTec, China. DNDI-6148 was incubated at 10 µM in the presence of Beagle dog and Sprague-Dawley rat liver microsomes (1 mg/mL) for 60 min. Following precipitation with a solution of formic acid and acetonitrile, metabolites were identified by LC-UV-MSn (n = 1–2).

**CYP Inhibition Studies.** The study was conducted by WuXi AppTec, Co., China, using the time-dependent (TDI) method. For detecting any IC₅₀ shift, the test compound (at concentrations of 0.05–50 µM) was first preincubated for 30 min at 37 °C with pooled human liver microsomes in the presence or not of NADPH. Following incubation with substrates (phenacetin for 1A2, diclofenac for 2C9, 5-(+) mephentoin for 2C19, dextromethorphan for 2D6, and midazolam or testosterone for 3A4), reactions were stopped by adding ice-cold acetonitrile, the samples were analyzed for the formation of metabolites by LC-MS/MS, and the percentage of inhibition was determined.

**hERG Inhibition.** An automated patch-clamp method (QPatch HTX) was used by WuXi AppTec, China. Chinese hamster ovary cells expressing hERG potassium channels were incubated at room temperature with test compounds (0.12, 0.37, 1.11, 3.33, 10, and 30 µM, in triplicate) or amitriptyline as a positive control.

**Plasma-Protein Binding.** Assays were performed by WuXi AppTec, Co., China. DNDI-6148 was incubated at 10 µM in solution in plasma (Balb/c mouse, golden (Syrian) hamster, Sprague-Dawley rat, Beagle dog or human) and dialyzed against 100 mM phosphate-buffered saline (pH 7.4) on a rotating plate incubated for 6 h at 37 °C.
HBSS buffer from A to B direction or B to A direction was determined in triplicate. Permeation of the compound to the basolateral side of the cell monolayer. The test compound, at a concentration of 2 μM, was determined by UV spectroscopy.

**Membrane Permeability.** Bidirectional permeability tests with MDCK-MDR1 cells were performed by WuXi AppTec, Co., China. The test compound, at a concentration of 2 μM, was determined by UV spectroscopy.

**Thermodynamic Solubility.** The thermodynamic solubility was measured by WuXi AppTec, China. All lots of the compound DMSO stock (10 mM) were transferred to fasted state simulated intestinal fluid (FaSSIF) buffer (pH 6.5) or fed state simulated intestinal fluid (FeSSIF) buffer (pH 5.0), and the mixtures were shaken for 24 h at room temperature. Following sampling by a Whatman filter device, the compound concentrations were determined by UV spectroscopy.

**Ames Assay.** Mini-Ames reverse mutation screens were conducted by WuXi AppTec, Co., China. Two Salmonella typhimurium strains (TA98 and TA100) were employed, both in the presence and absence of metabolic activation (induced rat liver S9). Test compounds were assessed at doses of 1.5, 4, 10, 25, 64, 160, 400, and 1000 μg per well, 2-aminanthracene, 2-nitrofluorene, and sodium azide were used as positive controls, and the negative control was DMSO.

**In Vivo Pharmacokinetic Studies.** In vivo pharmacokinetic studies were conducted at WuXi AppTec, Co., China. Compounds were formulated as a homogenous suspension in 2% ethanol, 1 mol equiv NaOH, dextrose (5% solution), and dosed twice p.o. on a single day (8 h apart, 25 or 50 mg/kg), or p.o. as a homogenous suspension (at 10 mg/kg). The K2-EDTA anticoagulant was added to blood samples collected at predose, 0.033 (i.v. only), 0.083 (p.o. only), 0.25, 0.5, 1, 3, 6, 9, and 24 h, and were stabilized with phosphoric acid (1% final) before analysis by LC-MS/MS. PK parameters were calculated using WinNonlin software (version 6.3).

**In Vivo Pharmacokinetic Studies.** In vivo pharmacokinetic studies were conducted at WuXi AppTec, Co., China. Treatments with LiOH hydrolyzed the ester function of the compound concentration. The investigators converted to DNDI-6148 (23-2) to convert to DNDI-6148 (23) by reaction with aniline 2, T3P, and base.

**Ethyl 5-Methyl-1-(pyridin-2-yl)-1H-1,2,3-triazole-4-carboxylate (23-3).** A solution of tetrazole[1,5-a]pyridine (23-2) (144 mg, 1.2 mmol) and ethyl 3-oxobutanoate (I) (1.56 g, 5.99 mmol) in EtOH (10 mL) and a solution of NaOEt (245 mg, 3.6 mmol) in EtOH (10 mL) were mixed in a 1:1 ratio and applied to the flow coil (Vapourtec RS-400 flow chemistry system). The reaction mixture was then heated at 150 °C with a residence time of 2 h. The crude product mixture was collected into a vial and acidified to pH 5 by the addition of HCl (2N aq.). The reaction mixture was then concentrated under reduced pressure and directly purified by silica flash column chromatography (10:90 → 50:50 EtOAc:hexane), affording ester 23-3 as a colorless solid (110 mg, 38% yield). 1H NMR (500 MHz, CDCl3) δ 8.59 (1H, d, J = 4.75 Hz), 8.00–7.93 (2H, m), 7.42 (1H, t, J = 6.03 Hz), 4.47 (2H, q, J = 7.12 Hz), 2.92 (3H, s), 1.45 (3H, t, J = 7.10 Hz). 13C NMR (126 MHz, CDCl3) δ 161.7, 150.2, 139.3, 139.1, 137.4, 121.4, 118.3, 61.0, 14.3, 11.0. HRMS (ESI+): m/z [M+H]+ calcd for C11H13N4O2, 233.1033; found, 233.1061.

**Lithium 5-Methyl-1-(pyridin-2-yl)-1H-1,2,3-triazole-4-carboxylate (II).** A solution of LiOH (1 M, aq, 0.31 mL, 0.31 mmol) was...
added to a solution of 23-ethyl 5-methyl-1-(pyridin-2-yl)-1H-1,2,3- triazole-4-carboxylate (23-3) (72 mg, 0.31 mmol) in THF/water (4:1, 10 mL), and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was then concentrated under reduced pressure, and the resultant aqeous residue was lyophlized to furnish the title compound as a colorless solid (64 mg, 97%).1H NMR (500 MHz, D2O) δ 7.78 (1H, d, J = 8.00 Hz), 7.79 (1H, d, J = 7.5, 3.53 Hz), 2.67 (3H, s). 13C NMR (126 MHz, D2O) δ 168.4, 149.0, 147.9, 141.9, 140.7, 137.6, 125.9, 120.6, 9.3. HRMS (ES+): m/z [M+H]+ calcd for C9H9N4O2, 205.0720; found, 205.0686.

DNDI-6148, N-(1-Hydroxy-1,3-dihydrobenzoc[1,2]oxaborol-6-yl)-5-methyl-1-(pyridin-2-yl)-1H-1,2,3-triazole-4-carboxamide (23). Neat DIPEA (130 mg, 1.28 mmol) was added to a stirred suspension of carboside II (45 mg, 0.214 mmol) and 6-aminobenzol[1,2] oxaborol-1(3H)-ol, hydrochloride (5) (59.6 mg, 0.321 mmol) in dry DME (3.0 mL) at room temperature. A solution of propylphosphonic anhydride (50% v/v in EtOAc, 0.757 mL, 0.642 mmol) was then added dropwise, and the resulting mixture was stirred for a further 16 h at room temperature. The reaction mixture was then concentrated under reduced pressure and directly purified by silica flash column chromatography (2.98 MeOH:CH2Cl2), affording the title compound as a colorless solid (21.7 mg, 95% purity, 29% yield). The product was further purified by reverse phase preparative HPLC (C18 silica, 5% → 95% MeCN:water + 0.1% NH3) to give an assay/analytical sample (64 mg, 97%).13C NMR (126 MHz, D2O) δ 168.4, 149.0, 147.9, 141.9, 140.7, 137.6, 125.9, 120.6, 9.3. HRMS (ES+): m/z [M+H]+ calcd for C74H70N11O5, 1053.4729; found, 1053.4712.

The title compound was a colorless solid (64 mg, 97%).1H NMR (500 MHz, D2O) δ 7.78 (1H, d, J = 8.00 Hz), 7.79 (1H, d, J = 7.5, 3.53 Hz), 2.67 (3H, s), 8.71 (1H, d, J = 1.76 Hz). 13C NMR (126 MHz, D2O) δ 159.8, 149.8, 149.7, 149.5, 140.5, 134.9, 138.1, 128.6, 127.5, 125.3, 123.0, 121.9, 119.5, 70.2, 10.7 (note, the signal for the benzene quaternary carbon atom directly linked to the boron atom was not observed in the 13C NMR due to severe broadening caused by the spin dynamics of the quadrupolar 11B nucleus). HRMS (ES+): m/z [M+H]+ calcd for C38H36N4O2, 578.2571; found, 578.2576.

In Vitro Drug Sensitivity Assays (MoA Studies). Drug sensitivity assays with wild-type and transgenic T. brucei and L. donovani cell lines were carried out as previously described.1,32 Confirmation of CPSF3 Overexpression Using MS. WT and L. donovani promastigotes (3 × 10^5) overexpressing CPSF3 were washed twice with PBS (1000 g, 4 °C, 5 min). The pellet was resuspended in 1.5 mL of lysis buffer (50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 1 mM DTT, and 1 × EDTA-free complete protease inhibitors (Roche)). Parasites were biologically inactivated by 3 × freeze/thaw cycles prior to lysis using a One-Shot Cell Disruptor (Constant Systems) at 30 kpsi. Lysed cells were then centrifuged (10 min, 4 °C, 15 000g), and the soluble material was harvested. Soluble cell lysates (100 μg samples) were reduced by the addition of tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 25 mM final concentration) and incubated at 37 °C for 10 min. The samples were then alkylated by the addition of iodoacetamide (25 mM final concentration) and incubated at RT for 1 h in the dark. Alkylated samples were digested by the addition of 1:50 endoprotease LysC (Wako, Japan) in 100 mM triethylammonium bicarbonate (TEAB), followed by incubation at 37 °C for 6 h, then the addition of 1:50 trypsin (1.50 dilution) and incubation at 37 °C overnight. The resulting digested peptides were then vacuum-dried.

LC-MS/MS Analysis. Analysis of peptides was performed on a Q-Exactive Plus mass spectrometer (Thermo Scientific) coupled to a Dionex Ultimate 3000 RSLC (Thermo Scientific). LC buffers used were as follows: Buffer A (0.1% formic acid in Milli-Q water (v/v)) and Buffer B (80% acetonitrile and 0.1% formic acid in Milli-Q water (v/v)). Aliquots (15 μL per sample) were loaded at 10 μL/min onto a PepMap nanoViper C18 column (100 μm × 2 cm, 5 μm, 100 Å, Thermo Scientific) equilibrated with 98% Buffer A. The column was washed for 5 min at the same flow rate and then was switched in line with a Thermofinnigan RSLC C18 column (75 μm × 50 cm, 2 μm, 100 Å, Thermo Scientific) equilibrated with 98% Buffer A and 2% Buffer B for 20 min and re-equilibrated in 2% Buffer B for 17 min prior to loading the next sample. The Q-Exactive Plus was used in the data-dependent mode. The scan cycle comprised MS1 scan (m/z range from 335 to 1600, with a maximum ion injection time of 20 ms, a resolution of 70 000, and an automatic gain control (AGC) value of 1 × 106), followed by 15 sequential dependent MS2 scans (with an isolation window set to 1.4 Da, resolution at 17 500, maximum ion injection time at 100 ms, and AGC 2 × 105). The stepped collision energy was set to 27 and fixed first mass to 100 m/z. Spectra were acquired in a centroid mode and unassigned charge states. Charge states above 6, as well as singly charged species, were rejected. To ensure mass accuracy, the mass spectrometer was calibrated on day 1 of the analyses. LC-MS analysis was performed by the FingerPrints Proteomics Facility (University of Dundee).

Data Analysis. MS data analysis was performed using MaxQuant software (http://maxquant.org/, version 1.6.2.6a). Carbamidomethyl (C), oxidation (M), acetyl (Protein N-term), deamidation (NQ), and Gln -> pyro-Glu were set as variable modifications. Proteins were identified by searching a protein sequence database containing T. brucei brucei TREU927 annotated proteins (downloaded from TriTrypDB, http://www.tritrypdb.org). LFQ and “match between runs” features were enabled. Trypsin/P and LysC/P were selected as the specific proteases with two potential missed cleavages. The FDR threshold for peptides and proteins was 0.01. FTMS MS/MS mass tolerance was set to 10 ppm, and FTMS MS/MS mass tolerance was set to 0.05 m/z.
Homology Modeling and In Silico Docking Studies. To identify suitable template structures for the generation of the L. donovani CPSF3 homology model, the sequence UniProtKB—E9BBR9 was used to query the PDB using “BLAST” as implemented in the NCBI blastp suite (https://blast.ncbi.nlm.nih.gov/). Endonucleases from five different species, human CPSF3 (PDB code 2I7T and 6MBQ—sequence identity 54%), Saccharomyces cerevisiae CPSF (PDB code 61ID—sequence identity 47%), Pyrococcus horikoshii CPSF3 (PDB code 3AF5—sequence identity 29%), Cryptosporidium hominis (PDB code 6Q5S—sequence identity 48%), and T. thermophila TTHA0252 (several structures available exemplified by PDB code 3JEM—sequence identity 30%), were identified as close analogues that could be used as template structures. The T. thermophila structure is complexed with a stable RNA analogue bound to the catalytic site located at the interface between the metallo-β-lactamase and β-CASP domains, whereas one human, S. cerevisiae and P. horikoshii structures are apo forms where the Zn2+ containing binding site is inaccessible. The C. hominis structure is in complex with a small boron-containing inhibitor (AN3661) and where the binding site is still inaccessible to a larger ligand. One of the human structures (6MBQ) is in complex with a small ligand that does not interact with the two Zn2+ ions and adopts the binding mode that causes structural rearrangements in loop regions adjacent to the binding cavity. Thus, the T. thermophila structure was selected as a suitable template. The alignment between the amino acid sequence of LdCPSF3 that of the T. thermophila CPSF3 was manually curated (Figure S5) and showed a sequence identity of 68% in the binding site area. A homology model for LdCPSF3 was built using the knowledge-based method in Prime in Schrödinger Release 2019-3: Schrödinger, LLC, New York, NY, 2020). Zinc atoms were modeled into the structure but not the RNA substrate. Due to its empty p-orbital, the boron atom in DNDI-6148 is a strong electrophile (Lewis acid) that can react with solvent water molecules. The nucleophilic attack of an activated water molecule on the trigonal boron atom leads to the formation of a tetrahedral negatively charged boron species. The three-dimensional structure of the hydroxylated form of DNDI-6148 was built in Maestro, minimized with the OPLS3 force field, and docked in the catalytic site of the LdCPSF3 model using GLIDE in Schrödinger. Docking results were subjected to a restrained minimization procedure with the OPLS3e force field, as implemented, where each heavy atom was allowed to deviate by up to 0.5 Å from its original position in the model.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c01437.

Chemistry experimental details for all compounds, Supplementary figures and tables related to MoA and docking studies; HPLC chromatograms of key compounds used in vivo; and data illustrating the PK properties of compound 23 in rat and dog and off-target profiling of compound 23 (PDF)

String data for all compounds (CSV)

DNDI-6148 rat and dog PK data (XLSX)

Compound 23 docked into LdCPSF3 (PDB)

AUTHOR INFORMATION

Corresponding Authors
Charles E. Mowbray — Drugs for Neglected Diseases initiative (DNDi), 1202 Geneva, Switzerland; orcid.org/0000-0003-3538-8116; Email: cmowbury@dndi.org

Susan Wyllie — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.; orcid.org/0000-0001-8810-5605; Email: s.wyllie@dundee.ac.uk

Authors
Stéphanie Braillard — Drugs for Neglected Diseases initiative (DNDi), 1202 Geneva, Switzerland
Paul A. Glossop — Sandexis Medicinal Chemistry Ltd, Kent CT13 9ND, U.K.; orcid.org/0000-0001-6567-5648
Gavin A. Whitlock — Sandexis Medicinal Chemistry Ltd, Kent CT13 9ND, U.K.
Robert T. Jacobs — Scynexis, Durham, North Carolina 27713, United States; orcid.org/0000-0001-9669-2862
Jason Speake — Scynexis, Durham, North Carolina 27713, United States
Bharathi Pandi — Scynexis, Durham, North Carolina 27713, United States
Bakela Nare — Scynexis, Durham, North Carolina 27713, United States
Louis Maes — Laboratory for Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, 2610 Wilrijk, Antwerp, Belgium
Vanessa Yardley — Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, U.K.
Yvonne Freund — Anacor Pharmaceuticals, Palo Alto, California 94303, United States
Richard J. Wall — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.
Sandra Carvalho — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.
Davide Bello — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.
Magali Van den Kerkhof — Laboratory for Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, 2610 Wilrijk, Antwerp, Belgium
Guy Caljon — Laboratory for Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, 2610 Wilrijk, Antwerp, Belgium
Ian H. Gilbert — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.; orcid.org/0000-0002-5238-1314
Victoriano Corpas-Lopez — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.
Iva Lukac — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.
Stephen Patterson — Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.
The authors gratefully acknowledge financial support for this work from U.K. aid, U.K.; the Bill & Melinda Gates Foundation; and the Federal Ministry of Education and Research (BMBF) through KiW, Germany. For its overall mission, DNDi also receives financial support from Médecins sans Frontières (MSF) International; and the Swiss Agency for Development and Cooperation (SDC), Switzerland. Work carried out at the University of Dundee was funded by the following Wellcome Trust grants: 105021, 203134/Z/16/Z, 218448/Z/19/Z.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
The DNDi thanks the staff of SCYNEXIS Inc., WuXi AppTech, LMPH, LSHTM, and the University of Dundee for their individual practical contributions to this work. The Mode of Action group, University of Dundee, would like to thank Professor David Horn and Dr. Eva Rico Vidal for allowing us to use their CRISPR-Cas9 edited cell line. In addition, they would like to thank the FingerPrints Proteomics Facility at the University of Dundee for assistance with proteomic studies.

ABBREVIATIONS USED
CC₅₀, half-maximum cytotoxic concentration; Clₐ₄₅, intrinsic clearance; CPSF3, cleavage and polyadenylation specificity factor subunit 3; DNDi, Drugs for Neglected Diseases initiative; FaSSIF, fasted state simulated intestinal fluid; FaSSIF, fasted state simulated intestinal fluid; HLM, human liver microsomes; HamLM, hamster liver microsomes; LAB, liposomal amphotericin B; MoA, mode of action; MRC5, liver microsomes; HamLM, hamster liver microsomes; LAB, liposomal amphotericin B; MoA, mode of action; MRC5, liver microsomes; HLM, human liver microsomes; SI, selectivity index; SSG, sodium stibogluconate; TTP, target product profile; VL, visceral leishmaniasis

REFERENCES
(1) Alvar, J.; Yactayo, S.; Bern, C. Leishmaniasis and poverty. Trends Parasitol. 2006, 22, 552–557.
(2) Alvar, J.; Vélez, I. D.; Bern, C.; Herrero, M.; Desjeux, P.; Cano, J.; Jannin, J.; den Boer, M. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 2012, 7, e35671.
(3) WHO Leishmaniasis fact sheet. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis.
(4) Fabio Zuccotto, Division of Biological Chemistry and Drug Discovery, Wellcome Centre for Anti-infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 SEH, U.K.; orcid.org/0000-0002-3888-7423
(5) Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.1c01437
(6) P.A.G., G.A.W., and S.W. prepared this manuscript on behalf of all the authors. All authors have given approval to the final version of the manuscript.
(7) Wylie, S.; Roberts, A. J.; Norval, S.; Patterson, S.; Foth, B. J.; Berriman, M.; Read, K. D.; Fairlamb, A. H. Activation of bicyclic nitro-drugs by a novel nitroreductase (NTR2) in Leishmania. PLoS Pathog. 2016, 12, e1005971.
(8) Wylie, S.; Thomas, M.; Patterson, S.; Crouch, S.; De Rycker, M.; Lowe, R.; Gresham, S.; Urbaniak, M. D.; Otto, T. D.; Stojanovski, L.; Simeons, F. R. C.; Manthri, S.; MacLean, L. M.; Zuccotto, F.; Homer, N.; Pflaumer, H.; Boesche, M.; Sastry, L.; Connolly, P.; Albrecht, S.; Berriman, M.; Dixone, G.; Gray, D. W.; Ghidelli-Disse, S.; Dixon, S.; Fiandor, J. M.; Wyatt, P. G.; Ferguson, M. A. J.; Fairlamb, A. H.; Miles, T. J.; Read, K. D.; Gilbert, I. H. Cyclin-dependent kinase 12 is a drug target for visceral leishmaniasis. Nature 2018, 560, 192–197.
(9) Wylie, S.; Brand, S.; Thomas, M.; De Rycker, M.; Chung, C. W.; Pena, I.; Bingham, R. P.; Bueren-Calduqui, J. A.; Cantiani, J.; Cebrian, D.; Crages, P. D.; Ferguson, L.; Gowami, P.; Hohrath, J.; Howe, J.; Jeacock, L.; Ko, E. J.; Korczynska, J.; MacLean, L. D.; Manthri, S.; Martinez, M. S.; Mata-Cantero, L.; Moniz, S.; Nihs, A.; Ounas-Cabello, M.; Pinto, E.; Riley, J.; Robinson, S.; Rowland, P.; Simeons, F. R. C.; Shishikura, Y.; Spinks, D.; Stojanovski, L.; Thomas, J.; Thompson, S.; Viayana Gaze, E.; Wall, R. J.; Zuccotto, F.; Horn, D.; Ferguson, M. A. J.; Fairlamb, A. H.; Fiandor, J. M.; Martin, J.; Gray, D. W.; Miles, T. J.; Gilbert, I. H.; Read, K. D.; Marco, M.; Wyatt, P. G. Preclinical candidate for the treatment of visceral leishmaniasis that acts through proteasome inhibition. Proc. Natl. Acad. Sci. U.S.A. 2019, 116, 9318–9323.
(10) Nagle, A.; Biggart, A.; Be, C.; Srinivas, H.; Hein, A.; Cardilha, D.; Scotti, R. J.; Pybus, B.; Kreishman-Deitrick, M.; Bursula, P.; Lai, Y. H.; Gao, M. Y.; Liang, F.; Mathison, C. J. N.; Liu, X.; Yeh, V.; Smith, J.; Lerario, N.; Xie, Y.; Chancellier, D.; Gibney, M.; Berman, A.; Chen, Y. L.; Jiricek, J.; Davis, L. C.; Liu, X.; Ballard, J.; Khare, S.; Eggimann, F. K.; Luneau, A.; Groesel, T.; Shapiro, M.; Richmond, W.; Johnson, K.; Rudowicz, P. J.; Rao, S. P. S.; Thompson, C.; Tunland, T.; Suppong, G.; Glyne, R. J.; Supel, F.; Wiesmann, C.; Molteni, V. Discovery and characterization of clinical candidate LXE408 as a kinetoplastid-selective proteasome inhibitor for the treatment of leishmaniasis. J. Med. Chem. 2020, 63, 10773–10781.
(11) AABBNI Acziborole factsheet. https://dndi.org/research-development/program/acoziborole/.
(12) Jacobs, R. T.; Nare, B.; Wring, S. A.; Orr, M. D.; Chen, D.; Sligar, J. M.; Jenks, M. X.; Noe, R. A.; Bowling, T. S.; Mercer, L. T.; Rewerts, C.; Gaulke, 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.; Don, R. SCYX-7158, an orally-active benzoxaborole for the treatment of stage 2 human African trypanosomiasis. PLoS Neglected Trop. Dis. 2011, 5, e1151.
(13) Nare, B.; Wring, S.; Bacchi, C.; Beaudet, B.; Bowling, T.; Brun, R.; Chen, D.; Ding, C.; Freund, Y.; Gaulke, E.; Hussain, A.; Jarnagin, K.; Jenks, 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.; Jacobs, R. Discovery of novel orally bioavailable oxaborole 6-carboxamides that demonstrate cure in a murine model of late-stage central nervous system african trypanosomiasis. Antimicrob. Agents Chemother. 2010, 54, 4379–4388.
(14) Van der Kerkhof, M.; Mabile, D.; Chatelain, E.; Mowbray, C. E.; Braillard, S.; Hendrickx, S.; Maes, L.; Caljon, G. In vitro and in vivo pharmacodynamics of three novel antileishmanial lead series. Int. J. Parasitol.: Drugs Drug Resist. 2018, 8, 81–86.
(15) Hann, M. M. Molecular obesity, potency and other addictions in drug discovery. In Multifaceted Roles of Crystallography in Modern Drug Discovery; Scapin, G.; Patel, D.; Arnold, E., Eds.; Springer: Dordrecht, 2015, pp 183–196.
(16) Peterson, L. A. Reactive metabolites in the biotransformation of molecules containing a furan ring. Chem. Res. Toxicol. 2013, 26, 6–25.
Wall, R. J.; Moniz, S.; Thomas, M. G.; Norval, S.; Ko, E. J.; Marco, M.; Miles, T. J.; Gilbert, I. H.; Horn, D.; Fairlamb, A. H.; Wyllie, S. Antitrypanosomal 8-hydroxy-naphthyridines are chelators of divalent transition metals. Antimicrob. Agents Chemother. 2018, 62, No. e00235-18.

Begolo, D.; Vincent, I. M.; Giordani, F.; Pöhner, I.; Witty, M. J.; Rowan, T. G.; Bengaly, Z.; Gillingwater, K.; Freund, Y.; Wade, R. C.; Barrett, M. P.; Clayton, C. The trypanocidal benzoxaborole AN7973 inhibits trypanosome mRNA processing. PLoS Pathog. 2018, 14, No. e1007315.

Wall, R. J.; Rico, E.; Lukac, I.; Zuccotto, F.; Elg, S.; Gilbert, I. H.; Freund, Y.; Alley, M. R. K.; Field, M. C.; Wyllie, S.; Horn, D. Clinical and veterinary trypanocidal benzoxaboroles target CPSF3. Proc. Natl. Acad. Sci. U.S.A. 2018, 115, 9616−9621.

Sonoiki, E.; Ng, C. L.; Lee, M. C.; Guo, D.; Zhang, Y. K.; Zhou, Y.; Alley, M. R.; Ahyong, V.; Sanz, L. M.; Lafuente-Monasterio, M. J.; Dong, C.; Schupp, P. G.; Gut, J.; Legac, J.; Cooper, R. A.; Gamo, F. J.; DeRisi, J.; Freund, Y. R.; Fidock, D. A.; Rosenthal, P. J. A potent antimalarial benzoxaborole targets a Plasmodium falciparum cleavage and polyadenylation specificity factor homologue. Nat. Commun. 2017, 8, No. 14574.

Bellini, V.; Swale, C.; Brienner-Pinchart, M. P.; Pezier, T.; Georgault, S.; Laurent, F.; Hakimi, M. A.; Bougdour, A. Target identification of an antimalarial oxaborole identifies AN13762 as an alternative chemotype for targeting CPSF3 in apicomplexan parasites. iScience 2020, 23, No. 101871.

Corpas-Lopez, V.; Moniz, S.; Thomas, M.; Wall, R. J.; Torrie, L. S.; Zander-Dinse, D.; Tinti, M.; Brand, S.; Stojanovski, L.; Manthri, S.; Hallyburton, I.; Zuccotto, F.; Wyatt, P. G.; De Rycker, M.; Horn, D.; Ferguson, M. A. J.; Clos, J.; Read, K. D.; Fairlamb, A. H.; Gilbert, I. H.; Wyllie, S. Pharmacological validation of N-myristoyltransferase as a drug target in Leishmania donovani. ACS Infect. Dis. 2019, 5, 111−122.

Van den Kerkhof, M.; Leprohon, P.; Mabille, D.; Hendrickx, S.; Tulloch, L. B.; Wall, R. J.; Wyllie, S.; Chatelain, E.; Mowbray, C. E.; Braillard, S.; Ouellette, M.; Maes, L.; Caljon, G. Identification of resistance determinants for a promising antileishmanial oxaborole series. Microorganisms 2021, 9, 1408.

Van Boeckelaer, K.; Cardiha, D.; Black, C.; Vesely, B.; Leed, S.; Scotti, R. J.; Wijnant, G. J.; Yardley, V.; Braillard, S.; Mowbray, C. E.; Ioset, J. R.; Croft, S. L. Novel benzoxaborole, nitroimidazole and anilimpyrazoles with activity against experimental cutaneous leishmaniasis. Int. J. Parasitol.: Drugs Drug Resist. 2019, 11, 129−138.

Stauber, L. A. Host Resistance to the Khartoum Strain of Leishmania donovani; The Rice University Pamphlet, 1958; Vol. 45, pp 80−96.

Jha, T. K.; Sundar, S.; Thakur, C. P.; Bachmann, P.; Karbwang, J.; Fischer, C.; Voss, A.; Berman, J. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. N. Engl. J. Med. 1999, 341, 1795−1800.

Jacobs, R. T. L. Y.; Scotti, R. J.; Robinson, S. J.; Mowbray, C. E.; Whitlock, G. A.; Speake, J. D.; Glossop, P. A.; Launay, D. F. M. WO2018160845 - Novel oxaborole analogues and uses thereof. 2018.

Wentrup, C. W.; Winter, H. W. Isolation of diazacycloheptatrienes from thermal nitrene-nitrene rearrangements. J. Am. Chem. Soc. 1980, 102, 6159−6161.

Beneke, T.; Madden, R.; Makin, L.; Valls, J.; Sunter, J.; Gluenz, E. A CRISPR Cas9 high-throughput genome editing toolkit for kinetoplastids. R. Soc. Open Sci. 2017, 4, No. 170095.

Beneke, T.; Gluenz, E. LeishGEdit: A method for rapid gene knockout and tagging using CRISPR-Cas9. In Methods in Molecular Biology; Humana Press: New York, NY, 2019; Vol. 1971, pp 189−210.

Jones, D. C.; Hallyburton, I.; Stojanovski, L.; Read, K. D.; Frearson, J. A.; Fairlamb, A. H. Identification of a κ-opioid agonist as a potent and selective lead for drug development against human African trypanosomiasis. Biochem. Pharmacol. 2010, 80, 1478−1486.

Wyllie, S.; Patterson, S.; Stojanovski, L.; Simeons, F. R.; Norval, S.; Kime, R.; Read, K. D.; Fairlamb, A. H. The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis. Sci. Transl. Med. 2012, 4, No. 119re1.