Identification of a 4-fluorobenzyl l-valinate amide benzoxaborole (AN11736) as a potential development candidate for the treatment of Animal African Trypanosomiasis (AAT)

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Animal African Trypanosomiasis (AAT) is a fatal, parasitic wasting disease of livestock and wild animals in Sub-Saharan Africa. It is caused primarily by the two protozoan parasites Trypanosoma congolense (T. congolense) and Trypanosoma vivax (T. vivax), which are transmitted by tsetse flies. AAT is responsible for 3 million cattle deaths annually and costs African livestock farmers approximately US$ 1–5 billion per year. The current standard-of-care for AAT is treatment with drugs, such as diminazene aceturate, isometamidium and homidamide, which are ineffective with drug resistance becoming an increasing concern. No new trypanocides have been approved for use in cattle for many years. Initial screening of the Anacor Pharmaceuticals library of novel benzoxaborole analogues demonstrated 100% efficacy with a single dose of 10 mg/kg against both T. congolense and T. vivax in mouse models of infection (IP dosing) and in the target animal, cattle, dosed intramuscularly. AN11736 has been advanced to early development studies.

Novel L-valinate amide benzoxaboroles and analogues were designed and synthesized for a structure-activity-relationship (SAR) investigation to optimize the growth inhibitory activity against Trypanosoma congolense (T. congolense) and Trypanosoma vivax (T. vivax) parasites. The study identified 4-fluorobenzyl (1-hydroxy-7-methyl-1,3-dihydrobenzo[\[1,2\]oxaborole-6-carbonyl]-L-valinate (5, AN11736), which showed IC50 values of 0.15 nM against T. congolense and 1.3 nM against T. vivax, and demonstrated 100% efficacy with a single dose of 10 mg/kg against both T. congolense and T. vivax in mouse models of infection (IP dosing) and in the target animal, cattle, dosed intramuscularly. AN11736 has been advanced to early development studies.

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and benzoxaborole 6-carboxylic acids (76). The general synthetic route is shown in Scheme 1. Reaction of alcohols (72) with N-Boc protected amino acids (73) gave ester intermediates (74), which were treated with dry hydrogen chloride to generate ester amine salts (75). Condensation of these amine salts (75) with benzoxaborole 6-carboxylic acids (76) provided the final compounds (1–71).

Scheme 2 illustrates the synthesis of 1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborole-6-carboxylic acid (83) as an example of key boron intermediates. Esterification of the acid (77) produced the ester (78), which was formulated to yield 79. Treatment of 79 with trifluoromethyl sulfonyl anhydride afforded the triflate compound (80), which was converted to the pinacol boron intermediate (81). Reduction of 81 and subsequent cyclization under aqueous acidic conditions generated the benzoxaborole ester (82). Hydrolysis of the ester group in 82 afforded the acid (83). The experimental procedures for the synthesis of 5 are described in the reference and note section.

Activity of compounds (1–71) against T. congolense and T. vivax was determined using the whole cell assays as described and their IC₅₀ values are summarized in Table 1. Lead compound 2 exhibited an IC₅₀ of 2 nM against both T. congolense and T. vivax. The 3,3-dimethyl analog 3 was essentially inactive (IC₅₀ = 2580 nM against T. c. and 9190 nM against T. v.) but better activity was observed for the 7-methyl analog 4 (IC₅₀ =}
Fig. 4. The difluoromethyl (IC50 values around 1 nM (see Table 1). We next explored variation of the substituent at the 7-position of benzoxaborole (spirocyclobutyl (vide infra). A wide range of substituents, such as halogens, trifluoromethyl, trifluoromethoxy, cyano and methylsulfonyl (5–19 in Fig. 3) on the benzyl ring were introduced to examine their effects on the antiparasitic activity. The majority of these fifteen compounds, with exception of 11 and 12, were very potent showing IC50 values around 1 nM (see Table 1). We next explored variation of the substituent at the 7-position of benzoxaborole (20–27 in Fig. 4). The difluoromethyl (22) and ethyl (24) analogs had similar activity to that of 5, but the electron-withdrawing fluoro (21) and trifluoromethyl (23) analogs were of significantly reduced potency. The amino acid linker was also modified (Fig. 5), with the cyclopropyl (28), 2-hydroxycyclopropyl (29), 2-fluorocyclopropyl (30) and spirocyclobutyl (32) analogs exhibiting potency similar to 5, but the spirocyclopropyl analog (31) exhibited decreased activity against T. v. parasitemia. Replacement of the 4-fluorophenyl in 5 with various heteroaryl groups (33–48, Fig. 6) resulted in the excellent activity in all cases except the NH-imidazole analog 44. Introduction of basic nitrogen-containing groups on the benzyl ester (49–54, Fig. 7) provided compounds 50–54 that were generally similar to 5. Lastly, aliphatic and heterocyclic esters (55–71, Fig. 8) were synthesized and many of these had IC50 values less than 1 nM as shown in Table 1.

Table 1

| Compound | IC50 (nM) | Compound | IC50 (nM) |
|----------|----------|----------|----------|
| T. c. | T. v. | T. c. | T. v. |
| 1 | 4.9 | 69 | 37 | 0.78 | 0.50 |
| 2 | 2.0 | 2.0 | 38 | 0.68 | 0.31 |
| 3 | 2580 | 9190 | 39 | 0.57 | 0.24 |
| 4 | 0.46 | 0.79 | 40 | 0.27 | 0.50 |
| 5 | 0.14 | 1.3 | 41 | 0.78 | 0.11 |
| 6 | 0.47 | 0.29 | 42 | 0.20 | 0.19 |
| 7 | 0.50 | 0.10 | 43 | 0.062 | NTa |
| 8 | 0.28 | 0.07 | 44 | 5000 | 980 |
| 9 | 0.18 | 0.10 | 45 | 0.37 | 0.33 |
| 10 | 0.22 | 2.7 | 46 | 0.42 | 0.24 |
| 11 | 0.16 | 0.24 | 47 | 0.20 | 0.081 |
| 12 | 0.31 | 19 | 48 | <-0.005 | 0.71 |
| 13 | 0.23 | 0.04 | 49 | 0.39 | 68 |
| 14 | 0.10 | 0.06 | 50 | 0.32 | 0.48 |
| 15 | 0.15 | 0.05 | 51 | 0.25 | 0.21 |
| 16 | 0.08 | 0.07 | 52 | 1.3 | 0.29 |
| 17 | 0.21 | 0.04 | 53 | 0.51 | 0.35 |
| 18 | 0.20 | 0.06 | 54 | 1.0 | 0.37 |
| 19 | 0.61 | 0.44 | 55 | 4.2 | 3.3 |
| 20 | 3.0 | 1.0 | 56 | 5.2 | 14 |
| 21 | 28 | NTa | 57 | 0.46 | 0.26 |
| 22 | 0.67 | 0.92 | 58 | 0.66 | 0.69 |
| 23 | 37 | 51 | 59 | 0.70 | 0.52 |
| 24 | 0.11 | 0.05 | 60 | <0.005 | 0.78 |
| 25 | 3.0 | 4.7 | 61 | 0.36 | 0.21 |
| 26 | 2.3 | 0.05 | 62 | 0.38 | 0.16 |
| 27 | 3.9 | 0.71 | 63 | 0.39 | 0.09 |
| 28 | 0.12 | 0.38 | 64 | 2.3 | 0.78 |
| 29 | 0.26 | 0.10 | 65 | 9.4 | 1.2 |
| 30 | 0.09 | 0.25 | 66 | 5.9 | 4.1 |
| 31 | 0.13 | 18 | 67 | 0.43 | 0.79 |
| 32 | 0.45 | 2.5 | 68 | 1.8 | 37 |
| 33 | 0.28 | 26 | 69 | 0.47 | 1.6 |
| 34 | 0.14 | 0.07 | 70 | 0.34 | 2.28 |
| 35 | 0.15 | 0.09 | 71 | 0.2 | 0.14 |
| 36 | 0.26 | 0.14 | | | |

* experimental procedures are described in the reference and note section.*

NTa = Not tested.

Scheme 2. Synthetic route for preparation of 83. Reagents and conditions: (a) H2SO4, EtOH, reflux, 24 h; (b) MgCl2, (CH2O)n, TEA, THF, reflux, 14 h; (c) Tf2O, pyridine, DMAP, DCM, 0–15 °C, 1 h; (d) Pin2B, KOAc, Pd(dppf)Cl2, 1,4-dioxane, N2, 85 °C, 15 h; (e) NaBH4, MeOH, THF, 0–15 °C, 1 h, then HCl, H2O; (f) NaOH, H2O, 40 °C, 3 h, then HCl for acidification.
Selected compounds were screened in both mouse and bovine in vitro metabolic stability assays (mouse S9 and bovine S9), as summarized in Table 2. These two species were chosen because the primary in vivo assays were conducted in mice, and the target animal of this research program is cattle. As shown in Table 2, out of 36 compounds tested, 27 compounds had \( C_{\text{LM}} < 10 \mu\text{M/min/mg protein} \) in both mouse and bovine S9 assays suggesting moderate to excellent in vitro metabolic stability. We evaluated the efficacy of selected compounds in two in vivo mouse models of infection, against T. conglense and T. vivax, respectively. Mice were infected with either 1 \( \times \) 10^7 T. c. parasites or 1 \( \times \) 10^8 T. v. parasites, and then treated with a test compound via intraperitoneal administration for 1, 2 or 4 consecutive days. The mice were then monitored for the presence of parasitemia for up to 60 days post treatment. We tested in T. c. model first, then followed up with T. v. for interesting compounds. As shown in Table 2, the 7-methyl analog 4 was superior to the 7-unsubstituted analog 2 in both T. c. and T. v. mouse models of infection. Of the 38 compounds tested with the in vivo mouse models, seven compounds (5, 8, 33, 34, 49, 62 and 71) demonstrated \( \geq 50\% \) curative efficacy in the T. c.-infected mouse model and 100% curative efficacy in the T. v.-infected mouse model, when tested as a single dose of 10 mg/kg. To select further from these seven compounds, four (5, 8, 33 and 71) had \( \geq 75\% \) curative efficacy in the T. c.-infected mouse model at a single dose of 10 mg/kg, and two (5 and 8) showed 100% curative efficacy. These two compounds were further tested at a single 5 mg/kg dose, but were unable to cure the T. c.-infected mice. We selected compound 5 (AN11736) to progress to exploratory studies to determine the efficacy and safety in a preliminary formulation against induced infections of T. vivax and T. conglense in cattle. AN11736 demonstrated 100% curative efficacy with a single intramuscular injection of 10 mg/kg against both T. conglense and T. vivax in cattle.

In summary, a novel series of 1,4-dinitrobenzene carbamates was discovered to be active against T. conglense and T. vivax, which are the main causative agents of Animal African Trypanosomiasis (AAT) in cattle. Two compounds (5 and 8) showed 100% curative efficacy in both T. c.- and T. v.-infected mice with a single dose of 10 mg/kg. Compound 5 (AN11736) demonstrated 100% curative efficacy with a single IM dose of 10 mg/kg against both T. conglense and T. vivax in cattle for a duration of 100 days. AN11736, as a novel chemical entity, was selected as a potential development candidate for the treatment of AAT.

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5. Experimental procedures for the synthesis of 4-fluorobenzyl (1-hydroxy-7-methyl-1,3-dihydrobenzo[e][1,2]oxaborole-6-carboxylic)-1-oxide (5, AN11736). To a solution of 77 (1.65 g, 10.8 mol) in ETOH (6.50 L) was added conc. H2SO4 (326 g, 3.25 mol). The reaction mixture was stirred at 85 \( ^\circ \text{C} \) for 24 h. TLC (thin layer chromatography) showed the starting material was consumed completely. The mixture was cooled to 15 \( ^\circ \text{C} \) and concentrated to 582 mmHg. The residue was poured into aqueous 2 M NaHCO3 (1.3 L) and the mixture was filtered. The filtrate was concentrated to give 78 (1.75 g, yield 90%) as a brown solid. AN11736 (400 MHz, CDCl3), \( \delta \): 7.41 (d, \( J = 7.9 \text{ Hz} \), 1H), 7.11 (t, \( J = 7.9 \text{ Hz} \), 1H), 6.94 (d, \( J = 7.9 \text{ Hz} \), 1H), 4.58 (br s, 1H), 4.37 (q, \( J = 7.4 \text{ Hz} \), 2H), 2.64 (s, 3H), 1.40 (t, \( J = 7.4 \text{ Hz} \), 3H). To a solution of 78 (80 g, 4.44 mmol) in THF (6.50 L) were added MgCl2(900 g, 4.32 mol) and (HCHO)2(600 g, 6.66 mol). The mixture was immediately heated to 90 \( ^\circ \text{C} \) and stirred for 14 h. The reaction mixture was cooled down to 15 \( ^\circ \text{C} \) and then added to a stirred solution of anhydrous H2O (3 L) and then 20 N HCl (1.5 L) slowly. The mixture was stirred for 0.5 h and then extracted with EtOAc. The combined organic phase was washed with sat. NaHCO3, dried over Na2SO4, filtered and concentrated under reduced pressure to give crude 79 (880 g) as a brown oil. AN11736 (400 MHz, CDCl3), \( \delta \): 11.40 (s, 1H), 9.93 (s, 1H), 7.46 (d, \( J = 7.6 \text{ Hz} \), 1H), 7.37 (d, \( J = 8.0 \text{ Hz} \), 1H), 4.40 (q, \( J = 7.4 \text{ Hz} \), 2H), 2.44 (s, 3H), 1.41 (t, \( J = 7.1 \text{ Hz} \), 3H). To a solution of 79 (900 g, 4.32 mol) in DCM (7.56 L) was added pyridine (1.02 kg, 12.9 mol) and DMAP (27 g, 221 mmol). The mixture was concentrated to 0 \( ^\circ \text{C} \) and TFA (1.56 mol) were added dropwise. The reaction mixture was warmed to 15 \( ^\circ \text{C} \) and stirred for 1 h. The mixture was quench to water (7.65 L) and then concentrated to give 80 (1.50 kg, 2.94 mol). The mixture was cooled to 5 \( ^\circ \text{C} \) and stirred for 1 h. The resulting mixture was filtered and concentrated under reduced pressure to give crude 81 (860 g) as a yellow oil. AN11736 (400 MHz, CDCl3), \( \delta \): 10.27 (s, 1H), 7.99 (s, 1H), 7.91–7.87 (m, 6H, 1H, 1H, 1H, 1H, 1H), 7.43 (s, 3H), 6.34 (s, 3H). A solution of 81 (1.00 kg, 2.94 mol), bis(pinacolato)diboron (1.12 kg, 4.41 mol) and KOAc (573 g, 5.84 mol) in 1,4-dioxane (6.50 L) was added Pd(dppf)Cl2, CuCl2, (150 g, 184 mmol). The mixture was stirred at 85 \( ^\circ \text{C} \) for 15 h under N2. The mixture was...
cooled to 15 °C, filtered and concentrated to give the crude product. The residue was purified by column chromatography (SiO2, petroleum ether/ethyl acetate = 40/1 to 4/1) to give crude 81 (842 g) as a yellow oil. To a solution of 81 (1.20 kg, 3.77 mol) in MeOH (300 mL) and THF (6 L) was added NaBH4 (80 g, 2.11 mol) in portions at 0 °C. Then the reaction mixture was stirred at 15 °C for 1 h. HPLC showed 81 was consumed completely. The reaction solution was adjusted to pH = 4 with 2 M HCl. The organic layer was removed in vacuum and the mixture was filtered. The cake was washed with petroleum ether (5 L) and dried in vacuum to give 82 (665 g, 80%) as a white solid. 1H NMR (400 MHz, DMSO-d6): δ 9.18 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 5.00 (s, 2H), 4.30 (q, J = 7.0 Hz, 2H), 2.68 (s, 3H), 1.33 (t, J = 7.0 Hz, 3H). To a mixture of 82 (867 g, 3.94 mol) in H2O (5 L) was added NaOH (394 g, 9.85 mol) in one portion. The solution was heated at 40 °C for 3 h. HPLC showed 82 was consumed completely. This batch was worked-up together with the other batches and acidified with 2 N HCl to pH = 2. The solid was filtered and washed with H2O (10 L). The cake was dried to give 83 (2.00 kg, yield 87%) as a white solid. 1H NMR (400 MHz, DMSO-d6): δ 12.75 (s, 1H), 9.13 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 4.98 (s, 2H), 2.68 (s, 3H); HPLC purity: 100% at both 220 nm and 254 nm; MS (ESI+): m/z = 12.4 Hz, 1H), 4.96 (s, 2H), 4.33 (t, J = 7.0 Hz, 2H), 2.68 (s, 3H); 2H), 2.13 (dd, J = 8.4 & 4.4 Hz, 1H), 2.42 (dd, J = 8.4 & 4.4 Hz, 1H), 2.13 (dd, J = 11.0 & 6.6 Hz, 1H), 1.08 (dd, J = 10.1 & 7.1 Hz, 6H). The crude product. The residue was purified by column chromatography (SiO2, petroleum ether/ethyl acetate = 50:1 to 10:1) to give 4-fluorobenzyl (84, 85% yield) as a white solid. 1H NMR (400 MHz, CDCl3): δ 8.4 Hz, 2H), 5.29–5.10 (m, 2H), 3.95 (br, s, 1H NMR (400 MHz, DMSO-d6)): δ 7.0 Hz, 3H) and 4-fluorobenzyl (72, R1 = 4-fluorobenzyl, 290 g, 2.30 mol) in petroleum ether/ethyl acetate = 50:1 to 10:1) to give 4-fluorobenzyl (84, 85% yield) as a white solid. 1H NMR (400 MHz, CDCl3): δ 8.0 Hz, 1H), 7.32 (d, J = 8.6 Hz, 2H), 5.19–5.08 (m, 2H), 5.01 (d, J = 8.6 Hz, 2H), 2.42 (s, 3H), 2.19–2.10 (m, 1H, 1.06 kg, 3.26 mol) in HCl/EtOAc (6.0 L) was stirred at 25 °C for 1 h. HPLC showed, indicating the presence of 1–5 trypanosomes per field, 2 indicating the presence of 6–20 trypanosomes per field and 3 indicating greater than 20 trypanosomes per field. Mice scoring 2 or 3 were immediately euthanized. After 60 days, parasitemic mice were considered cured. Untreated control mice survived on average for 10 and 6 days post infection for T. c. and T. v., respectively. All in vivo mouse experiments were conducted in accordance with the strict guidelines set out by the Swiss Federal Veterinary Office, under the ethical approval of license number #2813. 8. Method for testing compound efficacy in cattle: The cattle studies were conducted in accordance with the method described for cattle by Eisler et al. (2001) in studies conducted in fly-proof facilities and using T. congolense and T. vivax isolates that had previously been confirmed resistant in cattle to diminazine (7 mg/kg live weight) and/or isometamidium (1 mg/kg live weight). Cattle studies included negative (saline) controls, all assessments were made for 100 days post treatment (unless animals relapsed sooner) and were conducted by staff blinded (masked) to allocation of animals to treatment groups and in accordance with the principles of veterinary good clinical practice (VICH, 2000).