Benoxaborole Antimalarial Agents. Part 4. Discovery of Potent 6-(2-(Alkoxycarbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles

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Supporting Information

ABSTRACT: A series of 6-aryloxy benzoxaborole compounds was designed and synthesized for a structure–activity relationship (SAR) investigation to assess the changes in antimalarial activity which result from 6-aryloxy structural variation, substituent modification on the pyrazine ring, and optimization of the side chain ester group. This SAR study discovered highly potent 6-(2-(alkoxycarbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles (9, 27−34) with IC_{50} = 0.2−22 nM against cultured Plasmodium falciparum W2 and 3D7 strains. Compound 9 also demonstrated excellent in vivo efficacy against P. berghei in infected mice (ED_{90} = 7.0 mg/kg).

INTRODUCTION

Malaria represents a continuing public health problem for close to half of the world’s population. It is a parasitic infection that was responsible for an estimated 198 million clinical cases and 584000 deaths worldwide in 2013, mostly among children under the age of five.1 The most important causative parasite, Plasmodium falciparum, is transmitted to humans by infected mosquitoes and is responsible for most malarial severe morbidity and mortality. Current therapies to treat malaria caused by P. falciparum are heavily reliant on artemisinin-based combination therapies. However, emergence of resistance to the endoperoxide component of the combination has recently been identified, and resistance to older antimalarial drugs is already widespread.2−4 This situation has brought renewed urgency to discovering new medications that counter resistance and that are safe and easy for use in the most vulnerable populations.5−10

Boron-containing benzoxaboroles have shown potent activity against P. falciparum, with the best reported IC_{50} at 26 nM.11−14 A new scaffold with a 6-aryloxy benzoxaborole structural feature caught our attention from early library screening against P. falciparum. The screening identified a 6-(4-carboxyphenoxo)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (1 in Figure 1) with an IC_{50} of 120 nM. In a continuing effort to identify benzoxaboroles with better antimalarial in vitro and in vivo activities, we further explored structure–activity relationships (SARs) by varying the 6-aryloxy group (2−9 in Figure 1), changing substituents on the pyrazine ring (10−26) and exploring the pyrazinyl side chain ester group (27−35). These compounds have been synthesized and evaluated against P. falciparum. Representative compounds were further tested in a mouse model. Herein we report the synthesis and in vitro SAR of compounds 1−40 and safety, in vitro stability, pharmacokinetic, and in vivo efficacy results for selected compounds.

CHEMISTRY

Methods for the preparation of a diverse number of substituted benzoxaboroles have recently been published.15−22 The chemistry for the syntheses of compounds 1, 2, 6, 8, and 9 was described previously.23,24 Scheme 1 illustrates the simple displacement methods for the syntheses of compounds 3, 7, and 10. The synthesis of benzoxaborole 36 was reported previously,24 and intermediate esters 37 and 38 were easily prepared from the corresponding commercial carboxylic acids. The methods for the syntheses of compounds 4 and 5 are shown in Scheme 2. Nucleophilic substitution of a fluorine atom in 41 by the hydroxyl group in 40 generated 42, followed by esterification, giving the methyl ester 43. Catalytic boronylation converted the bromo compound 43 to boron compound 44. Reduction of the aldehyde of 44 followed by
Acidification provided the ester benzoxaborole 5, which was hydrolyzed to the acid compound 4.

As shown in Scheme 3, acid compound 45 was converted to the amide 46 followed by dehydration, resulting in cyano compound 47. Displacement of a chlorine atom in 47 by the phenoxy moiety in compound 36 gave the desired compound 11, which was reduced to generate compound 13, containing the aminomethyl side chain. The nitrile compound 11 was also converted to 23 and 26, with the carboximidamide and tetrazole side chain, respectively. Reduction of the ester in 9 afforded the hydroxymethyl analogue 12.

The chemistry for the syntheses of compounds 14 and 22 is shown in Scheme 4. The carboxylic acid of 45 was converted to the ester 48, which was reduced to give the aldehyde compound 49. A coupling reaction of intermediate 49 with 36 generated 50, which led to the amine compound 14 by reductive amination and the oxime analogue 22 by reacting with hydroxylamine.

Scheme 5 illustrates the syntheses for compounds 15–21. Amide intermediates 46 and 51–56 were obtained by converting the acid 45 to its isobutylcarbonyl anhydride or its carbonyl chloride followed by reacting with the corresponding amines or sulfonamide. Finally, compounds 15–21 were generated by coupling the amides 46 and 51–56 with 6-hydroxy benzoxaborole 36.

The chemistry for the preparation of compounds 24 and 35 is shown in Scheme 6. The carboxylic acid group in 45 was converted to the amide of 57, which was then cyclized to 58. The chlorine atom in 58 was replaced by the hydroxyl benzoxaborole 36 to give 24. Ring-opened ester product 35 was also obtained because of the hydrolysis of the oxazoline ring under higher temperature.

For the synthesis of compound 25, the chemistry is illustrated in Scheme 7. From a common intermediate 48, compound 59 was obtained by hydrazine exchange with the ester methoxy group. Further acetylation of 59 gave 60, which was cyclized to generate 61. The substitution reaction between 61 and 36 provided the final product 25.

Syntheses of ester compounds 27–34 are shown in Scheme 8. The carboxylic acid in 45 was transformed to the corresponding ester intermediates 62–69, which underwent a substitution reaction with the common intermediate 6-hydroxy benzoxaborole 36 to provide the final ester compounds 27–34.

**RESULTS AND DISCUSSION**

**Structure–Activity Relationships.** The in vitro inhibitory activities of compounds 1–35 against the 3D7 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *P. falciparum* are shown in Figure 1. As shown in Scheme 3, acid compound 45 was converted to the amide 46 followed by dehydration, resulting in cyano compound 47. Displacement of a chlorine atom in 47 by the phenoxy moiety in compound 36 gave the desired compound 11, which was reduced to generate compound 13, containing the aminomethyl side chain. The nitrile compound 11 was also converted to 23 and 26, with the carboximidamide and tetrazole side chain, respectively. Reduction of the ester in 9 afforded the hydroxymethyl analogue 12.

The chemistry for the syntheses of compounds 14 and 22 is shown in Scheme 4. The carboxylic acid of 45 was converted to the ester 48, which was reduced to give the aldehyde compound 49. A coupling reaction of intermediate 49 with 36 generated 50, which led to the amine compound 14 by reductive amination and the oxime analogue 22 by reacting with hydroxylamine.

Scheme 5 illustrates the syntheses for compounds 15–21. Amide intermediates 46 and 51–56 were obtained by converting the acid 45 to its isobutylcarbonyl anhydride or its carbonyl chloride followed by reacting with the corresponding amines or sulfonamide. Finally, compounds 15–21 were generated by coupling the amides 46 and 51–56 with 6-hydroxy benzoxaborole 36.

The chemistry for the preparation of compounds 24 and 35 is shown in Scheme 6. The carboxylic acid group in 45 was converted to the amide of 57, which was then cyclized to 58. The chlorine atom in 58 was replaced by the hydroxyl benzoxaborole 36 to give 24. Ring-opened ester product 35 was also obtained because of the hydrolysis of the oxazoline ring under higher temperature.

For the synthesis of compound 25, the chemistry is illustrated in Scheme 7. From a common intermediate 48, compound 59 was obtained by hydrazine exchange with the ester methoxy group. Further acetylation of 59 gave 60, which was cyclized to generate 61. The substitution reaction between 61 and 36 provided the final product 25.

Syntheses of ester compounds 27–34 are shown in Scheme 8. The carboxylic acid in 45 was transformed to the corresponding ester intermediates 62–69, which underwent a substitution reaction with the common intermediate 6-hydroxy benzoxaborole 36 to provide the final ester compounds 27–34.
falciparum were determined (Table 1). Compounds 1−9 (see Figure 1) were designed to examine the effects of the left-side aromatic moiety, carboxylic acid (COOH) and ester (COOR) groups on antimalarial activity. Compound 1, containing a COOH group, showed submicromolar (0.12 μM) activity against the W2 strain, whereas its ester analogue 2 was without apparent antimalarial activity. Addition of a nitrogen atom ortho-to-oxygen bridge in compound 3 did not improve activity. However, when the nitrogen was moved to the position ortho to the carboxyl functional group, the acid compound 4 had submicromolar activity against W2 and 3D7 strain parasites, and its ester analogue 5 was about 5-fold more active. With two nitrogens, both meta to the carboxyl functional group, compound 6, containing a pyrimidine ring, had decreased activity. When one of the two nitrogens was moved to the position ortho to the carboxyl functional group, compound 7, with a pyridazine ring, showed excellent activity. When the left-side aromatic ring was changed to a pyrazine ring with one nitrogen ortho to the carboxyl functional group and the other ortho to the oxygen bridge, the acid compound 8 was somewhat less active, whereas its ester compound 9 was extremely potent, with low nanomolar IC50 values.

With the identification of the pyrazine ring, compounds 10−26 were designed and synthesized to investigate the effects of left-side substituent variation on antimalarial activity. As shown in

**Scheme 2. Synthesis of Compounds 4 and 5**

Reagents and conditions: (a) Cs2CO3, DMF, 80 °C, 16 h; (b) SOCl2, MeOH, 0−60 °C, 16 h; (c) Pd(PPh3)4Cl2, KOAc, 1,4-dioxane, N2, 100 °C, 0.5 h; (d) NaBH4, MeOH, 0 °C to rt, 1 h, then 1 N HCl; (e) LiOH·H2O, IPA: H2O (4:1), 40 °C, 1 h, then 1 N HCl.

**Scheme 3. Synthesis of Compounds 11−13, 23, and 26**

Reagents and conditions: (a) (1) SOCl2, DCM, 50 °C, 16 h, (2) NH4OH; (b) (CNCl)3 DMF, rt, 1 h; (c) intermediate 36, Cs2CO3, DMF, 50 °C, 2 h; (d) LiAlH4, THF, 0 °C, 50 min; (e) NH2OH·HCl, Na2CO3, H2O, 40 °C, 2 h; (f) NaN3, NH4Cl, DMF, rt, 1 h; (g) LiAlH4, THF, 0 °C, 50 min.

**Scheme 4. Synthesis of Compounds 14 and 22**

Reagents and conditions: (a) SOCl2, MeOH, 0−60 °C, 16 h; (b) DIBAL-H, THF, −78 °C, 2 h; (c) intermediate 36, Cs2CO3, DMF, 50 °C, 2 h; (d) Me2NH·HCl, NaBH(OAc)3, DCM, rt, 16 h; (e) NH2OH·HCl, NaOH, EtOH, rt, 3 h.
The compounds varied widely. Oxazoline and oxadiazole groups are critical for potent activity. Therefore, several other ester compounds (27–35) were designed and synthesized to further explore the effect of ester side chain variation on antimalarial activity. Linear and primary esters such as n-butyl ester 30 had outstanding activity, which was better than that of tertiary and more hindered analogues such as t-butyl ester (31). Ester compound 35, containing a terminal amine group on the ester side chain, had decreased potency, presumably because the charged amino group decreased permeability into parasites.

**Cytotoxicity Results.** Representative compounds were selected for in vitro cytotoxicity tests using human Jurkat cells (Table 2). In general, the tested compounds showed low cytotoxicities against this cell line.

**In Vitro Stability and Pharmacokinetic Profiles of 8, 9, and 15.** Stability testing showed that the amide 15 was stable in both mouse and human liver microsomes, but the ester 9 had poor stability (Table 3). However, because of the excellent in vitro potency of the ester 9, we planned to investigate its in vivo efficacy. Therefore, 9 and two closely related compounds, 8 (an acid) and 15 (an amide), were selected for pharmacokinetic profiling. The acid 8 had high plasma concentration, good exposure, and good bioavailability after oral administration in mice (Table 3). The ester 9 was bioavailable after oral dosing and was extensively metabolized to the acid 8 in vivo, resulting in higher plasma concentration and exposure of the metabolite acid 8 compared to the parent ester 9. An amide functional group is more stable than an ester upon hydrolysis in vivo. Indeed, the amide 15 showed improved plasma concentration, exposure, half-life, and bioavailability compared to 9. Production of the in vivo metabolite 8 from the amide 15 was minor, with <1% conversion.

**In Vivo Efficacy in a P. berghei Mouse Model.** Four compounds (4, 8, 15, and 9) were tested in a P. berghei mouse model. These compounds were selected for testing based on their chemical structural features, in vitro activities, physicochemical properties, and pharmacokinetic results. Compound 4 has a carboxylic acid group linked to a pyridine ring. Compound 8 has a pyrazine ring replacing the pyridine ring in 4. These compounds showed similar in vitro activities. Compounds 15 and 9 are a carboxamide and a carboxylic methyl ester of 8, respectively. Compounds were dosed by oral administration once a day for 4 consecutive days, beginning 1 h after infection by intraperitoneal administration of P. berghei-infected erythrocytes. The acids 4 and 8 and the amide 15 offered good in vivo efficacy. The ester 9 was the most effective.
Table 1. In Vitro Activity Results for Compounds 1–35 against Cultured *P. falciparum*<sup>a</sup>

| entry | cLogD<sub>7.4</sub> | IC<sub>50</sub> (nM)<sup>a</sup> (W2) | IC<sub>50</sub> (nM)<sup>a</sup> (3D7) | entry | cLogD<sub>7.4</sub> | IC<sub>50</sub> (nM) (W2) | IC<sub>50</sub> (nM) (3D7) |
|--------|-----------------|-----------------|-----------------|--------|-----------------|-----------------|-----------------|
| 1      | –0.37           | 120 ± 10        | 1090 ± 170 (72 h) | 20     | –0.53           | 6660 ± 1350     | >5000 ± 0       |
| 2      | 3.60            | 0%              | nt<sup>d</sup>   | 21     | –0.25           | 190 ± 10        | 130 ± 10        |
| 3      | 2.98            | 0%              | nt<sup>d</sup>   | 22     | 1.33            | 1770 ± 470      | 3000 ± 720      |
| 4      | –1.64           | 170 ± 10        | 540 ± 60         | 23     | 0.01            | >100000 ± 0     | >5000 ± 0       |
| 5      | 1.74            | 23 ± 2          | 120 ± 0          | 24     | 1.76            | 800 ± 70        | 1460 ± 210      |
| 6      | 1.80            | 80%<sup>c</sup> | nt<sup>d</sup>   | 25     | –0.21           | >100000 ± 0     | >5000 ± 0       |
| 7      | 1.11            | 82 ± 16         | 100 ± 40         | 26     | 1.31            | >100000 ± 0     | >5000 ± 0       |
| 8      | –2.35           | 150 ± 10        | 300 ± 40 (72 h)  | 27     | 1.47            | 0.5 ± 0.008     | 4.4 ± 1         |
| 9      | 1.13            | 1.4 ± 0.3       | 1.9 ± 0          | 28     | 1.88            | 1.2 ± 0.1       | 1.4 ± 0         |
| 10     | 1.18            | 9380 ± 270      | >5000 ± 0        | 29     | 2.34            | 0.5 ± 0.007     | 2.5 ± 0         |
| 11     | 1.39            | 470 ± 80        | 3250 ± 490       | 30     | 2.33            | 0.2 ± 0.04      | 0.7 ± 0         |
| 12     | 0.50            | 440 ± 20        | 2240 ± 240       | 31     | 1.96            | 19 ± 1          | 22 ± 2         |
| 13     | –0.27           | 110 ± 6         | 470 ± 30         | 32     | 2.67            | 0.4 ± 0.07      | 2 ± 0          |
| 14     | 1.12            | >100000 ± 0     | >5000 ± 0        | 33     | 2.71            | 0.7 ± 0.001     | 2.9 ± 0         |
| 15     | 0.23            | 210 ± 50        | 350 ± 130        | 34     | 2.85            | 6.6 ± 2         | 8.9 ± 2        |
| 16     | 0.48            | 240 ± 30        | 320 ± 50         | 35     | –0.24           | 190 ± 50        | 250 ± 40        |
| 17     | 0.82            | 120 ± 8         | 870 ± 220        | 36     | 19.5            | 21 ± 6          | na<sup>a</sup>  |
| 18     | 0.72            | 150 ± 10        | 960 ± 30         | 37     | 0.16            | 0.6 ± 0.1       | na<sup>a</sup>  |
| 19     | –0.27           | >100000 ± 0     | 5900 ± 3200      | 38     | 0.92            | 0.09 ± 0.001    | 0.99 ± 0.13     |

<sup>a</sup>Experimental procedures for the in vitro assays for the W2 and 3D7 strains are described in Supporting Information. IC<sub>50</sub> values were determined from two independent replicates for W2 strain and three replicates for 3D7 strain, and standard deviations were calculated. Artemisinin and chloroquine were used as reference controls for W2 strain assay, and atovaquone was used for 3D7 assay. Compounds were incubated with parasites for 48 h unless otherwise noted.<sup>c</sup> cLogD<sub>7.4</sub> was calculated using ChemAxon software under the condition of pH = 7.4. The value shown is percentage inhibition at 5 μM. <sup>d</sup>Not tested. <sup>e</sup>Not applicable.

Table 2. In Vitro Cytotoxicity Results for Representative Compounds<sup>a</sup>

| compd | IC<sub>50</sub> (µM, Jurkat cell) | compd | IC<sub>50</sub> (µM, Jurkat cell) |
|--------|---------------------------------|--------|---------------------------------|
| 5      | >100                            | 27     | 16.8                            |
| 8      | >100                            | 28     | >100                            |
| 9      | 7.59                            | 29     | 17.6                            |
| 15     | 48.2                            | 30     | >100                            |
| 17     | 79.9                            | 31     | >100                            |
| 18     | >100                            | 33     | 52.4                            |

<sup>a</sup>The experimental procedure for the in vitro cytotoxicity assay was described previously.<sup>11</sup>

Efficacious among the four compounds tested with ED<sub>90</sub> = 7.0 mg/kg (Table 4). Considering survival of experimental animals, control mice reached lethal parasitemia within 6–8 days. All four tested compounds extended survival, with modest benefits from compound 4, additional benefit with compounds 8 and 15, and the greatest efficacy with the ester 9 (Table 4).

Physicochemical properties such as lipophilicity, in vitro potency, and pharmacokinetics were assessed. The acids, 4 and 8, have –1.64 and –2.35 cLogD<sub>7.4</sub> values, respectively, indicating that the carboxylic group is negatively charged (COO<sup>–</sup>), likely resulting in inefficient penetration of parasite-infected erythrocytes. The amide 15 has improved lipophilicity and PK profile, but its in vitro activity was not high. The ester 9 was extremely active in vitro and its lipophilicity (cLogD<sub>7.4</sub> = 1.13) suggests good cell penetration,<sup>25</sup> which may contribute importantly to its low in vivo ED<sub>90</sub>. However, the metabolic instability and less favored PK parameters of 9 need to be further addressed. Our goal is to improve PK parameters while maintaining desired physicochemical properties of 9.

Table 3. In Vitro Stability in Liver Microsomes and Pharmacokinetic Parameters for 8, 9, and 15 in Mice<sup>a</sup>

| stability in liver microsome | oral PK parameters | intravenous PK parameters |
|-----------------------------|--------------------|----------------------------|
| compd | % remain at 1 h | % remain at 1 h | % remain at 1 h | % remain at 1 h | C<sub>max</sub> (µg/mL) | T<sub>max</sub> (h) | AUC<sub>0→inf</sub> (h·µg/mL) | T<sub>1/2</sub> (h) | F (%) | CL (mL/h/kg) | V<sub>ss</sub> (mL/kg) | AUC<sub>0→inf</sub> (h·µg/mL) | T<sub>1/2</sub> (h) |
| 8 (an acid)<sup>b</sup> | nd<sup>a</sup> | nd | nd | nd | 19.5 | 0.25 | 8.62<sup>f</sup> | 0.23 | 67 | 2326 | 848 | 4.3 | 1.1 |
| 9 (an ester)<sup>b</sup> | 0 | 0 | 0 | 0 | 0.16 | 0.25 | 0.10 | 0.1 | 23 | 12463 | 926 | 0.4 | 0.3 |
| 8 from 9<sup>d</sup> | 0.92 | 0.25 | 0.61 | 1.5 | 29 | 0.99 | 0.13 |
| 15 (an amide)<sup>c</sup> | 0.92 | 0.25 | 0.61 | 1.5 | 29 | 0.99 | 0.13 |

<sup>a</sup>Abbreviations: C<sub>max</sub>, maximum concentration of drug in plasma; T<sub>max</sub>, time to maximum concentration of drug in plasma; AUC<sub>0→inf</sub>, area under the curve extrapolated to infinity; T<sub>1/2</sub>, terminal half-life; F, oral bioavailability; CL, clearance; V<sub>ss</sub>, volume of distribution at steady state. Oral dosing at 30 mg/kg and intravenous dosing at 10 mg/kg. Oral dosing at 5 mg/kg and intravenous dosing at 5 mg/kg. The acid 8 detected from studies of the ester 9 because there was conversion of 9 to 8 in vivo. Not determined. This is AUC<sub>0→24h</sub>.
The control group had 100% survival on day 4 and 0% on day 5. In the experiment, which was stopped on day 18, mice in the chloroquine (CQ) control group had 100% survival on day 18 and those in the vehicle had 60% survival on day 4 after the onset of therapy.

**EXPERIMENTAL SECTION**

**Starting Materials and Solvents**. Starting materials and solvents purchased from commercial sources were used without further purification except where noted. High performance liquid chromatography (HPLC) was used to determine the purity of the compounds synthesized. The data confirmed that the target compounds generally had ≥95% of purity with exceptions where noted. Proton (1H) NMR spectra were recorded at room temperature on Bruker 300 or 400 instruments (Bruker Corporation, Billerica, Massachusetts, USA) using DMSO-d6 as solvent. Chemical shifts are given in parts per million (ppm). Electrospray ionization–mass spectrometry (ESI–MS) and LC–MS were carried out on an API2000 (AB Sciex, Framingham, Massachusetts, USA) or a Finnigan LCQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). HPLC was conducted on an Agilent 1200 system (Agilent Technologies, Santa Clara, California, USA) using a BDS Hypersil C-18 column (150 mm × 4.6 mm, 5 μm, 120 nm pore size, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The mobile phase used was a mixture of water (containing 0.1% phosphoric acid) and buffer B (CH3CN). The column was eluted with a gradient of 95% buffer A and 5% buffer B over 10 min, followed by 40% buffer A and 60% buffer B for 1 min, followed by a gradient over 1 min to 95% buffer A and 5% buffer B that was maintained for three more minutes at a flow rate of 1.0 mL/min with a column temperature of 30 °C and UV detection at 220 and 254 nm. Silica gel 60 nm (200–425 mesh, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used for flash column chromatography. Pharmacokinetic study with experimental animals was designed and performed with reference to the guidelines of the Institutional Animal Care and Use Committee (Anacor Pharmaceuticals, Inc., AN-7-2007) and reviewed by members of the committee. The in vivo efficacy work with experimental animals was performed in accordance with the institutional guidelines as defined by the Institutional Animal Care and Use Committee (UCSF, AN109984).

**Methods for Testing in Vitro Antimalarial Activity**. W2 strain P. falciparum parasites were cultured in human erythrocytes and RPMI-1640 culture media with either 10% human serum or 0.5% Albumax serum substitute under 3% O2, 5% CO2, and 92% N2. Parasites synchronized to ring stage by treatments with 5% n-sorbitol were cultured with serial dilutions of benzoxaboroles from 5 to 10 mM stocks in 96-well microplate cultures including 200 μL of media/well, 2% hematocrit, and 1% parasitemia. At the completion of 48 h incubations, when untreated cultures contained new rings, parasites were fixed with 2% formaldehyde for 48 h, and 5 μL aliquots were transferred to another 96-well plate containing 150 μL/well of staining solution (100 mM NH4Cl, 0.1% Triton X-100, and 1 mM YOYO-1 in

| Compound | Oral Dosage | d4 | d5 | d6 | d7 | d8 | d9 | d10 | d11 | d12 | d13 | d14 | d15 | d16-d19 | d20 | ED90 (mg/kg) |
|----------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|-----------|----|-------------|
| 4        | 100 mg/kg   | 100| 100| 100| 100| 80 | 0  |    |    |    |    |    |    |           |    | 21.1       |
|          | 33 mg/kg    | 100| 100| 100| 100| 60 | 0  |    |    |    |    |    |    |           |    |            |
|          | 11 mg/kg    | 100| 100| 100| 100| 40 | 0  |    |    |    |    |    |    |           |    |            |
|          | 3 mg/kg     | 100| 100| 100| 60 | 20 | 0  |    |    |    |    |    |    |           |    |            |
|          | 1 mg/kg     | 100| 100| 100| 60 | 0  |    |    |    |    |    |    |    |           |    |            |
| 8        | 100 mg/kg   | 100| 100| 100| 100| 60 | 0  |    |    |    |    |    |    |           |    | 30.3       |
|          | 33 mg/kg    | 100| 100| 100| 100| 40 | 40 | 0  |    |    |    |    |    |           |    |            |
|          | 11 mg/kg    | 100| 100| 100| 100| 60 | 20 | 20 | 20 |    |    |    |    |           |    |            |
|          | 3 mg/kg     | 100| 100| 100| 60 | 20 | 0  |    |    |    |    |    |    |           |    |            |
|          | 1 mg/kg     | 100| 100| 100| 80 | 40 | 0  |    |    |    |    |    |    |           |    |            |
| 9        | 100 mg/kg   | 100| 100| 100| 100| 100| 60 | 40 | 40 | 20 | 0  |    |    |           |    | 7.0        |
|          | 33 mg/kg    | 100| 100| 100| 100| 80 | 80 | 0  |    |    |    |    |    |           |    |            |
|          | 11 mg/kg    | 100| 100| 100| 100| 80 | 80 | 0  |    |    |    |    |    |           |    |            |
|          | 3 mg/kg     | 100| 100| 100| 100| 80 | 40 | 40 | 40 |    |    |    |    |           |    |            |
|          | 1 mg/kg     | 100| 100| 100| 100| 100| 40 | 0  |    |    |    |    |    |           |    |            |
| 15c      | 200 mg/kg   | 100| 100| 100| 100| 100| 40 | 0  |    |    |    |    |    |           |    | 38.7       |
|          | 100 mg/kg   | 100| 100| 100| 100| 100| 60 | 0  |    |    |    |    |    |           |    |            |
|          | 33 mg/kg    | 100| 100| 100| 100| 100| 60 | 0  |    |    |    |    |    |           |    |            |
|          | 11 mg/kg    | 100| 100| 100| 100| 100| 60 | 0  |    |    |    |    |    |           |    |            |
|          | 3 mg/kg     | 100| 100| 100| 80 | 60 | 0  |    |    |    |    |    |    |           |    |            |
|          | 1 mg/kg     | 100| 100| 100| 60 | 0  |    |    |    |    |    |    |    |           |    |            |
| CQ 30 mg/kg | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 20 | NA 

**CONCLUSION**

In summary, we identified and characterized a benzoxaborole chemical series that was highly potent against *P. falciparum*. Carboxylic ester 6-(2-alkoxycarbonyl)pyrazinyl-5-oxo)-1,3-di-hydro-1-hydroxy-2,1-benzoxaboroles (9, 27–34) demonstrated potent in vitro activity, with IC50 values of 0.2 to 22 nM against cultured parasites. Compound 9 also showed excellent in vivo efficacy (ED90 = 7.0 mg/kg).

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PBS). Parasites per erythrocyte were then determined by flow cytometry from plots of forward scatter against fluorescence (excitation 488 nm, emission 520 nm) using a FacSort flow cytometer (Beckton Dickinson) equipped with an AMS Loader (Cytek Development). All values were normalized to percent control activity, and IC50s were calculated using the Prism 3.0 program (GRAPHPAD Software). Goodness of fit was assessed by R2 values, and mean, drug dose—response curves yielded R2 values >0.95.

Inhibition of 3D7 strain parasites was assessed using the [3H]-hypoxanthine incorporation assay. Briefly, parasites were cultured in human erythrocytes (from the Spanish Red Cross blood bank) using RPMI-1640 culture media (Gibco) supplemented with 0.5% Albumax II (Invitrogen), 2% d-treheose (Sigma-Aldrich), 0.3% glutamine (Sigma-Aldrich), and 5 mM hypoxanthine (Sigma-Aldrich). Cultures were maintained at 37 °C at an atmosphere of 5% O2, 5% CO2, and 95% N2. To assess inhibition, asynchronous parasite cultures with 0.5% parasitemia and 2% hematocrit were exposed to 3-fold serial dilutions of test compounds for 24 h in 96-well plate cultures (Costar no. 3894).

After 24 h, [3H]-hypoxanthine was added, plates were incubated for an additional 24 h, and parasites were harvested on glass fiber filters (Wallac no. 1450-421) using a Cell Harvester 96 (TOMTEC, PerkinElmer). Filters were dried on scintillator sheets (MeltiLex A, PerkinElmer) to determine incorporation of [3H]-hypoxanthine. Radioactivity was measured using a microbeta counter (PerkinElmer). Data are normalized using the incorporation of the positive control (infected erythrocytes without drug). IC50 values were determined using Excel and Graf. IC50 values were determined from at least three independent experiments, and standard deviations were calculated.

Method for Testing in Vivo Stability in Liver Microsomes. The aliquot of benzoxaborole sample stock solution (1 mg/mL in DMSO) was added to a 96-well plate containing mouse or human liver microsomes in the presence or absence of NADPH. The final concentrations of benzoxaborole, NADPH, and liver microsomes were 1 μM benzoxaborole, 1 mM NADPH, and 0.5 mg/mL microsomal protein in 100 mM potassium phosphate buffer (pH 7.4), and the total volume was 200 μL per well. All samples were tested in duplicate. One set of preincubation samples (absolute zero) of benzoxaborole was initially stored at −80 °C during the incubation period to rule out thermal instability and served as a control for the initial concentration. Incubations of benzoxaborole in liver microsomes without NADPH served as negative controls, while incubations of liver microsomes only, with or without NADPH, served as background controls for metabolite identification experiments. The samples were incubated at 37 °C in a shaking water-bath, and samples were obtained at 0, 15, 30, 60, and 120 min. As a positive control, dextromethorphan prepared with stock stock solution was incubated in the presence of NADPH. The final concentration of dextromethorphan was 5 μM, and the total incubation volume was 200 μL per well. The samples were incubated at 37 °C in a shaking water-bath, and the sampling times were 0, 15, 30, 60, and 120 min. All incubations were terminated by addition of 600 μL of cold acetonitrile. An additional 80 μL of cold acetonitrile containing the internal standard at 1 μg/mL was then added. The samples were vortexed for 10 min and centrifuged for 20 min at approximately 4000 rpm. The supernatants were analyzed by LC/MS/MS.

Method for Pharmacokinetics Measurement. Adult female CD-1 mice received the benzoxaborole test material either by intravenous injection or by oral gavage in formulated solutions. For benzoxaborole, the doses were 10 mg/kg for iv and 30 mg/kg for oral, and the formulation used 100% saline. For benzoxaborole, the dose was 5 mg/kg for both iv and oral, and the formulation used polyethylene glycol 400, dimethylacetamide, and water (ratio 45/10/ 45, pH = 5.8). For benzoxaborole, the dose was also 5 mg/kg for both iv and oral, and the formulation used polyethylene glycol 300, propylene glycol, and water (ratio 55/25/20, pH = 5.9). Blood samples were collected and analyzed for drug content using HPLC coupled to tandem mass spectrometry.

Method for Testing in Vivo Efficacy Using a P. berghei Mouse Model. Female Swiss Webster Mice (average of 20 g body weight) were infected by the intraperitoneal injection of 6 × 106 Plasmodium berghei-infected erythrocytes collected from a previously infected animal. Mice were treated once daily by oral gavage with various concentrations of test compounds, beginning 1 h after initial infection, for 4 consecutive days. All compounds were formulated in a vehicle composed of polyethylene glycol 300, propylene glycol and water (weight ratio, 55/25/20), and administered in a total volume of 100 μL. Beginning on day 4 after infection, parasitemias were counted daily by examination of Giemsma stained smears. All counts within a single experiment were performed by the same investigator.

The ED90 of the compounds was defined as the mean dose (mg/kg; 5 mice per dose tested) that reduced parasitemia by 90% compared to that in vehicle treated controls 4 days after the initiation of therapy. The ED90 was estimated using nonlinear regression analysis with Graphpad Prism 5 software. When parasitemias exceeded 50%, animals were euthanized. Chloroquine (30 mg/kg) was used as a positive control. Animal survival and morbidity was monitored for 21 days post-infection.

Ethyl 6-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)nicotinate (3). To a solution of 36 (1.2 g, 8.0 mmol) in 1,4-dioxane (100 mL) was added KHMDS (48 mL, 0.5 M in toluene, 3 equiv) at 0 °C. The reaction was stirred at room temperature (rt) for 15 min, and then intermediate 3 (2.97 g, 16 mmol) was added at 0 °C. The reaction mixture was stirred at 80 °C for 22 h and quenched by adding cold brine at 0 °C. The solution pH was adjusted to 3 with 1 N HCl, and the mixture was extracted with ethyl acetate (EA). The organic phase was separated, washed with brine, dried over anhydrous Na2SO4, and filtered. The residue after rotary evaporation was purified by column chromatography (silica gel, DCM:MeOH = 40:1, v/v) to the final desired product 3 (0.109 g, yield 4.6%) as a light-yellow solid. 1H NMR (300 MHz, DMSO-d6): δ 9.22 (s, 1H), 8.68 (s, 1H), 8.32 (d, 1H), 7.50–7.45 (m, 2H), 7.32 (d, 1H), 7.17 (d, 1H), 5.01 (s, 2H), 4.35 (q, 2H), 1.35 (t, 3H) ppm. HPLC area: 98.2% at 220 nm and 99.3% at 254 nm. MS: m/z = 300 (M + 1, ES+).

Methyl 5-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)nicotinate (5) and 5-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)picolinic Acid (4). To a solution of 40 (3.0 g, 21.56 mmol, 1.1 equiv) in DMSO (33 mL) were added 41 (4.0 g, 19.61 mmol) and Cs2CO3 (8.1 g, 58.83 mmol, 3 equiv) in one portion at rt. The reaction mixture was stirred at 80 °C overnight. After completion, the reaction mixture was poured into 2 N HCl (300 mL) and extracted with EA (3 × 100 mL). The organic phases were washed with brine, dried over MgSO4, filtered, and evaporated to a solid (4). The residue was purified by column chromatography to the desired product 42 as a white solid (4.0 g, 63% yield). To a solution of 42 (6.3 g, 19.56 mmol) in MeOH (12 mL) was added SOCl2 (3.6 g, 48.90 mmol, 2.5 equiv) under N2. The reaction was heated to 60 °C and stirred overnight. The mixture was evaporated to a residue, which was poured into NaHCO3 (50 mL) and extracted with EA (3 × 20 mL). The combined organic phases were washed with brine, dried over Na2SO4, filtered, and evaporated to 43 as solid (3.8 g, 93% yield). To a solution of 43 (0.8 g, 3.38 mmol), Pin2B2 (0.9 g, 3.57 mmol, 1.5 equiv), and KOAc (0.7 g, 7.14 mmol, 3 equiv) in 1,4-dioxane (12 mL) was added Pd(PPh3)2Cl2 (170 mg, 0.24 mmol, 0.1 equiv) under N2. The reaction was heated at 100 °C for 0.5 h. Then the reaction was cooled to rt, poured into water (100 mL), and extracted with EA (3 × 3 mL). The combined organic phases were washed with brine, dried over Na2SO4, filtered, and evaporated to a residue. The residue was purified by silica gel column (PE:EA = 5:1) to the desired product 44 (0.5 g, 62% yield) as an oil. To a solution of 44 (0.4 g, 1.04 mmol) in MeOH (5.2 mL) was added NaBH4 (40 mg, 1.04 mmol, 1 equiv) in portions at 0 °C. The reaction was stirred at rt for 1 h. The reaction was quenched with 1 N HCl (4 mL), stirred for another 0.5 h, and extracted with EA (3 × 20 mL). The combined organic phase was washed with brine, dried over Na2SO4, filtered, and evaporated. The residue was purified by column chromatography to the desired product 5 as white solid (0.2 g, 71% yield). 1H NMR (300 MHz, DMSO-d6): δ 6.92 (s, 1H), 8.50 (d, 1H), 8.07 (d, 1H), 7.54–7.32 (m, 4H), 5.02 (s, 2H), 3.86 (s, 3H) ppm. HPLC purity: 99.6% at

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220 nm and 99.3% at 254 nm. Mass: m/z = 286.1 (M + 1, ESI+). To a solution of S (0.3 g, 1.05 mmol) in 2-propanol (8.9 mL) was added LiOH·H₂O (88 mg, 2.10 mmol, 2 equiv) in H₂O (2.6 mL) in one portion at 0 °C. The reaction mixture was stirred at 40 °C for 2 h. The reaction was quenched with 1 N HCl (2 mL) and extracted with EA (3 × 5 mL). The combined organic phase was washed with brine, dried over Na₂SO₄, filtered, and evaporated. The residue was purified by column chromatography to the desired product S as a white solid (0.2 g, 74% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 8.74 (s, 1H), 8.43 (s, 1H), 8.17 (s, 1H), 7.86−7.54 (m, 2H), 7.26 (d, 1H), 5.46 (t, 1H), 4.98 (s, 2H), 4.55 (d, 2H) ppm. HPLC area: 94% at 254 nm. Mass: m/z = 259 (M + H, ESI+).

5-(Aminomethyl)pyrazin-2-ylbenzo[c][1,2]oxaborol-1(3H)-ol Hydrochloride (13). To a solution of LiAlH₄ (285 mg, 7.5 mmol, 3 equiv) in THF (8 mL) was added a solution of 11 (630 mg, 2.5 mmol, 1 equiv) in THF (4.5 mL). The reaction mixture was stirred at 0 °C for 50 min, diluted with H₂O (5 mL), dissolved with MeOH (50 mL), and filtered. The residue after rotary evaporation was purified by column chromatography to a solid. The solid was stirred with 4 N HCl/Methanol and then concentrated to the desired solid product 13 (22 mg, 27% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 9.28 (br s, 1H), 8.63 (s, 1H), 8.42 (br s, 3H), 8.30 (s, 1H), 7.51−7.49 (m, 2H), 7.30 (d, 1H), 5.02 (s, 2H), 4.19 (q, 2H) ppm. HPLC area: 97.5% at 220 nm. Mass: m/z = 258 (M + H, ESI+).

5-((Dimethylamino)methyl)pyrazin-2-ylbenzo[c][1,2]oxaborol-1(3H)-ol (14). A solution of 13 (1.58 g, 10 mmol) in SOCl₂ (33.3 mL) was stirred at 80 °C for 2 h and then concentrated under reduced pressure. MeOH (20 mL) was added and stirred at 50 °C for 2 h. The mixture was concentrated in vacuo. The residue was mixed with HCl·H₂O (30 mL) and extracted with EA (2 × 10 mL). The organic phases were combined, washed with brine, dried over MgSO₄, filtered, and evaporated. The residue was purified by column chromatography on silica gel (EA:PE = 10%) to the methyl ester 14 (1.4 g, 81% yield). To a solution of 14 (500 mg, 2.9 mmol) in THF (26 mL) was added DIBAL·H (9 mL, 9 mmol, 3.1 equiv) dropwise at −78 °C with good stirring. The reaction was stirred at −78 °C for 2 h, poured into water (300 mL), and acidified with 1 N HCl to pH = 3. The resulted solution was extracted with EA (2 × 10 mL). The organic phases were combined, washed with brine, dried over MgSO₄, filtered, and evaporated. It was purified by column chromatography on silica gel (EA:PE = 20%) to 49 (205 mg, 49% yield). To a solution of 49 (105 mg, 0.733 mmol, 1.1 equiv) and 36 (100 mg, 0.667 mmol) in DMF (2.2 mL) was added Cs₂CO₃ (434 mg, 1.33 mmol, 2 equiv). The reaction mixture was stirred at 50 °C for 1 h, poured into water, and extracted with EA (2 × 20 mL). The organic phases were washed, dried over MgSO₄, filtered, and evaporated. The residue was then washed with n-hexane and dried in vacuo to the product 50 (110 mg, 64% yield). A solution of 50 (60 mg, 0.234 mmol) and dimethylamine hydrochloride (23 mg, 0.281 mmol, 1.2 equiv) in DCM (1.2 mL) was stirred at rt for 1 h. Then NaBH(OAc)₃ (198 mg, 0.94 mmol, 4 equiv) was added, and the reaction mixture was stirred at rt overnight. It was triturated with DCM (20 mL), filtered, and the filtrate concentrated. The residue was purified by column chromatography on silica gel (MeOH:DCM = 10%) to the product 14 (38 mg, 56% yield). ²H NMR (300 MHz, DMSO-d₆): δ 9.21 (s, 1H), 8.51 (s, 1H), 8.19 (s, 1H), 7.49−7.47 (m, 2H), 7.31 (d, 1H), 5.01 (s, 2H) ppm. HPLC area: 98.1% at 220 nm and 97.4% at 254 nm. Mass: m/z = 286 (M + H, ESI+).

5-(Hydroxymethyl)pyrazin-2-ylbenzo[c][1,2]oxaborol-1(3H)-ol (12). To a suspension of LiAlH₄ (342 mg, 9 mmol, 3 equiv) in THF (7 mL) was added dropwise a solution of 9 (860 mg, 3.0 mol L⁻¹ in THF (5 mL). The reaction mixture was stirred at 0 °C for 50 min, diluted with H₂O (40 mL), adjusted to pH = 3 with 1 N HCl, and extracted with EA (2 × 50 mL). The organic phases were combined, washed with brine, dried over anhydrous Na₂SO₄, and filtered. The residue after rotary evaporation was purified by column chromatography to the product 12 (210 mg, 27% yield). ¹H NMR (300 MHz, DMSO-d₆): δ 9.17 (s, 1H), 8.43 (s, 1H), 8.17 (s, 1H), 7.46−7.43 (m, 2H), 7.26 (d, 1H), 5.46 (t, 1H), 4.98 (s, 2H), 4.55 (d, 2H) ppm. HPLC area: 94% at 254 nm. Mass: m/z = 259 (M + H, ESI+).

5-(Hydroxymethyl)pyrazin-2-ylbenzo[c][1,2]oxaborol-1(3H)-ol Hydrochloride (15). A solution of 12 (516 mg, 3 mmol) in DCM (6 mL) was stirred at 50 °C for 2 h. It was poured into 1 N HCl (100 mL) and extracted with EA (4 × 100 mL). The combined organic phase was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by column chromatography to the desired product 15 (460 mg, 56% yield) as a solid. ²H NMR (300 MHz, DMSO-d₆): δ 9.24 (s, 1H), 8.70 (s, 1H), 8.67 (s, 1H), 8.08 (br s, 1H), 7.71 (br s, 1H), 7.52−7.49 (m, 2H), 7.35 (dd, 1H), 5.02 (s, 2H) ppm. HPLC area: 98.1% at 220 nm and 98.8% at 254 nm. Mass: m/z = 272 (M + H, ESI+).

5-(Hydroxymethyl)pyrazin-2-ylbenzo[c][1,2]oxaborol-1(3H)-ol-N-methylpyrazine-2-carboxamide (16). This compound was prepared by the similar method described above for the synthesis of 15. ²H NMR (300 MHz, DMSO-d₆): δ 9.24 (s, 1H), 8.72−8.70 (m, 1H),
Ethyl 5-(1-Hydroxy-1,3-dihydrobenzo[1,2]oxaborol-6-yl)oxy)pyrazin-2-ylamide (18). This compound was prepared by the similar method described above for the synthesis of 15. 1H NMR (300 MHz, DMSO-d6): δ 9.29 (s, 1H), 8.69 (s, 1H), 8.66–8.61 (m, 2H), 7.51–7.49 (m, 2H), 7.36 (dd, 1H), 5.03 (s, 2H), 4.36–4.33 (m, 2H). HPLC area: 97.8% at 220 nm and 99.0% at 254 nm. Mass: m/z = 311 (M + 1, ESI+).

N-(2-Hydroxyethyl)pyrazine-2-carboxamide (19). This compound was prepared by the similar method described above for the synthesis of 15. 1H NMR (300 MHz, DMSO-d6): δ 9.63 (br s, 1H), 9.27 (s, 1H), 9.05 (t, 1H), 8.73 (s, 1H), 8.65 (s, 1H), 7.53–7.50 (m, 2H), 7.35 (d, 1H), 5.03 (s, 2H), 3.62–3.68 (m, 2H), 2.81 (s, 6H) ppm. HPLC area: 99.5% at 220 nm and 99.6% at 254 nm. Mass: m/z = 343 (M + 1, ESI+).

N-(1-Hydroxy-1,3-dihydrobenzo[1,2]oxaborol-6-yl)-N-(isopropylsulfonyl)pyrazine-2-carboxamide (20). This compound was prepared by the similar method described above for the synthesis of 15. 1H NMR (300 MHz, DMSO-d6): δ 11.87 (br s, 1H), 9.25 (s, 1H), 8.76 (s, 1H), 8.65 (s, 1H), 7.54–7.50 (m, 2H), 7.37 (dd, 1H), 5.03 (s, 2H), 3.78 (septet, 1H), 1.33 (d, 6H) ppm. HPLC area: 96.7% at 220 nm and 99.7% at 254 nm. Mass: m/z = 378 (M + 1, ESI+).

(E)-5-(1-Hydroxy-1,3-dihydrobenzo[1,2]oxaborol-6-yl)pyrazine-2-carbaldehyde oxime (21). To a solution of 50 mg (100 mg, 0.005 mol) and hydroxyamine hydrochloride (31 mg, 0.05 mmol, 1.14 equiv) in EtOH (1.36 mL) was added sodium hydroxide solution (3N, 0.26 mL). The reaction mixture was stirred at rt for 2 h, poured into 2 N HCl (100 mL), and extracted with EA (2 mL). The organic phase was washed with brine, dried over MgSO4, filtered, and concentrated to the desired product (12 mg, 50% yield). 1H NMR (300 MHz, DMSO-d6): δ 11.17 (s, 1H), 9.25 (s, 1H), 8.76 (s, 1H), 8.65 (s, 1H), 7.54–7.50 (m, 2H), 7.37 (dd, 1H), 5.03 (s, 2H), 3.78 (septet, 1H), 1.33 (d, 6H) ppm. HPLC area: 94.6% at 220 nm and 98.8% at 254 nm.

(Z)-N-Hydroxy-5-(1-hydroxy-1,3-dihydrobenzo[1,2]oxaborol-6-yl)pyrazine-2-carboxamide (22). To a solution of 11 (200 mg, 0.75 mmol) in DMF (2 mL) were added NaN3 (86 mg, 1.27 mmol, 1.7 equiv) and NaN3 (82 mg, 1.27 mmol, 1.7 equiv). The reaction mixture was stirred at rt for 2 h, poured into 2 N HCl (100 mL) and brine (15 mL), dried over anhydrous Na2SO4, and filtered. The residue after rotary evaporation was purified by column chromatography to the desired product 26 (40 mg, 0.01 mmol, 1.2 equiv). 1H NMR (300 MHz, DMSO-d6): δ 9.32 (s, 1H), 9.06 (s, 1H), 8.73 (s, 1H), 7.54–7.49 (m, 2H), 7.36 (dd, 1H), 5.01 (s, 2H), 2.60 (s, 3H) ppm. HPLC area: 96.8% at 220 nm and 95.5% at 254 nm. Mass: m/z = 311 (M + H, ESI+) and 333 (M + Na).

5-(1-Hydroxy-1,3-dihydrobenzo[1,2]oxaborol-6-yl)-N-propargylpyrazine-2-carboxamide (24). To a solution of 5 (632 mg, 4 mmol), 2-amino-2-methylpropan-1-ol (430 mg, 4.8 mmol, 1.2 equiv), HOBt (702 mg, 5.2 mmol, 1.3 equiv), and EDC (1g, 5.2 mmol, 1.3 equiv) in DMF (40 mL), 25 (4 mg, 0.01 mmol, 1 equiv) was added TEA (1.2g, 12 mmol, 3 equiv). The reaction was stirred at rt for 30 min, and a normal workup resulted in the desired product 27 (670 mg, 80% yield) as a white solid. To SOCl2 (6 mL) was added 57 (400 mg, 1.65 mmol), and the solution was stirred at rt for 2 h. The reaction solution was concentrated to dryness, and the residue was dissolved in DCM (30 mL), adjusted to pH 8–9 with saturated NaHCO3 and then separated. The organic phase was washed with brine, dried over MgSO4, and filtered. To a solution of 58 (85 mg, 0.04 mmol) in DCM (4 mL) were added 36 (60 mg, 0.04 mmol, 1 equiv) and Cs2CO3 (325 mg, 1 mmol, 2.5 equiv) at rt. The reaction mixture was stirred at 60 °C for 2 h, poured into 2 N HCl (100 mL), and extracted with EA (3 × 50 mL). The combined organic phase was washed with brine, dried over MgSO4, and filtered. To a solution of 59 (100 mg, 0.08 mmol) in DCM (5 mL) was added AcCl (46 mg, 0.58 mmol, 1 equiv) dropwise. The mixture was stirred at rt for 1 h, diluted with H2O (10 mL), and extracted with DCM (20 mL). The organic phase was washed with 1 N HCl (10 mL), IN NaHCO3 (15 mL), and brine (15 mL), dried over anhydrous Na2SO4, and filtered. The residue after rotary evaporation was purified by column chromatography to the desired product 60 (95 mg, 76% yield). The solution of 60 (90 mg, 0.02 mmol, 1 equiv) in POCl3 (1.8 mL) was stirred at 110 °C for 0.5 h. After completion, the reaction was diluted with ice water (10 mL), washed with IN NaHCO3 (10 mL), and extracted with EA (15 mL). The organic phase was washed with brine (10 mL), dried over anhydrous Na2SO4, and filtered. The residue after rotary evaporation was purified by column chromatography to the desired product 61 (62 mg, 76% yield). To a solution of 61 (60 mg, 0.0306 mmol) in DMF (1 mL) were added Cs2CO3 (251 mg, 0.765 mmol, 2.5 equiv) and 36 (46 mg, 0.0306 mmol, 1 equiv). The reaction mixture was stirred at 80 °C for 45 min, diluted with H2O (10 mL), and extracted with EA (10 mL). The organic phase was washed with brine (10 mL), dried over anhydrous Na2SO4, and filtered. The residue after rotary evaporation was purified by column chromatography to the desired product 25 (35 mg, 50% yield). 1H NMR (300 MHz, DMSO-d6): δ 9.25 (s, 1H), 8.84 (s, 1H), 8.73 (s, 1H), 7.54–7.49 (m, 2H), 7.36 (dd, 1H), 5.01 (s, 2H), 2.60 (s, 3H) ppm. HPLC area: 96.8% at 220 nm and 95.5% at 254 nm. Mass: m/z = 311 (M + H, ESI+) and 333 (M + Na).
mmol) in SOCl₂ (42 mL) was stirred at 80 °C for 2 h and then concentrated directly. The residue was dissolved with EtOH (40 mL) and then stirred at 50 °C for 2 h. The reaction was evaporated to dryness, poured into H₂O (40 mL), and extracted with EA (3 × 50 mL). The combined organic phase was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo to give the ethyl ester 62 (2.0 g, 85% yield). The solution of 62 (205 mg, 1.1 mmol), 36 (150 mg, 1 mmol, 1 equiv) and Cs₂CO₃ (815 mg, 2.5 mmol, 2.5 equiv) in DMF (3.3 mL) was stirred at 50 °C for 1 h. After completion, the reaction was poured into water (30 mL), adjusted to pH = 3 with 2 N HCl, and extracted with EA (3 × 20 mL). The combined organic phase was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuum. The residue was purified by column chromatography to the desired product 27 (55 mg, 25% yield).¹ H NMR (500 MHz, DMSO-d₆): δ 9.24 (s, 1H), 8.76 (s, 1H), 8.67 (s, 1H), 7.54–7.50 (m, 2H), 7.37 (dd, 1H), 5.03 (s, 2H), 4.36 (q, 2H), 1.33 (t, 3H) ppm. HPLC area: 95.8% at 220 nm and 95.6% at 254 nm. Mass: m/z = 301 (M + H, ES⁺).

**Isopropyl 5-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (31).** This compound was prepared by following the methodology similar to that described above for 27.¹ H NMR (500 MHz, DMSO-d₆): δ 9.23 (s, 1H), 8.74 (s, 1H), 8.65 (s, 1H), 7.53–7.50 (m, 2H), 7.36 (dd, 1H), 5.02 (s, 2H), 4.11 (d, 2H), 2.06–2.00 (m, 1H), 0.97 (d, 6H) ppm. HPLC area: 98.3% at 220 nm and 97.4% at 254 nm. Mass: m/z = 351 (M + Na, ES⁺).

**n-Butyl 5-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (30).** This compound was prepared by following the methodology similar to that described above for 27.¹ H NMR (500 MHz, DMSO-d₆): δ 9.24 (s, 1H), 8.74 (s, 1H), 8.65 (s, 1H), 7.51–7.47 (m, 2H), 7.34 (dd, 1H), 5.01 (s, 2H), 4.30 (t, 2H), 1.72–1.63 (m, 2H), 1.45–1.33 (m, 2H), 0.90 (t, 3H) ppm. HPLC area: 95.6% at 220 nm and 93.2% at 254 nm. Mass: m/z = 351 (M + Na, ES⁺).

**34**: 1H NMR (300 MHz, DMSO-d₆): δ 9.27 (s, 1H), 9.04 (s, 1H), 8.70 (s, 1H), 8.23 (br s, 3H), 7.54–7.48 (m, 2H), 7.35 (d, 1H), 5.01 (s, 2H), 4.32 (s, 2H), 1.33 (s, 6H) ppm. HPLC area: 97.6% at 220 nm and 97.8% at 254 nm. Mass: m/z = 344 (M + H, ES⁺).

|| ASSOCIATED CONTENT | SUPPORTING INFORMATION |
|---|---|

**Figures illustrating mice survival and efficacy EDₙ₀ data.** The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00678.

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Notes
The authors declare no competing financial interest.

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**ABBREVIATIONS USED**

NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography; ESI, electrospray ionization; DMSO, dimethyl sulfoxide; KHMDS, potassium hexamethyldisilazide; EA, ethyl acetate; DMF, dimethylformamide; PE, petroleum ether; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; c-CPBA, meta-chloroperbenzoic acid; HOBt, hydroxybenzotriazole; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NMM, N-methylmorpholine

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