Journal of Medicinal Chemistry

Article

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Benzoxaborole 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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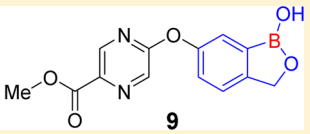
Supporting Information

ABSTRACT: A series of 6-hetaryloxy 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}s = 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).

IC_{50} = 1.4 nM (*P.f.* W2 strain)
1.9 nM (*P.f.* 3D7 strain)
ED_{90} = 7.0 mg/kg
in *P. b.* - infected mice



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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-carboxyphenoxy)-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

Received: May 1, 2015

DOI: 10.1021/acs.jmedchem.5b00678
J. Med. Chem. XXXX, XXX, XXX−XXX

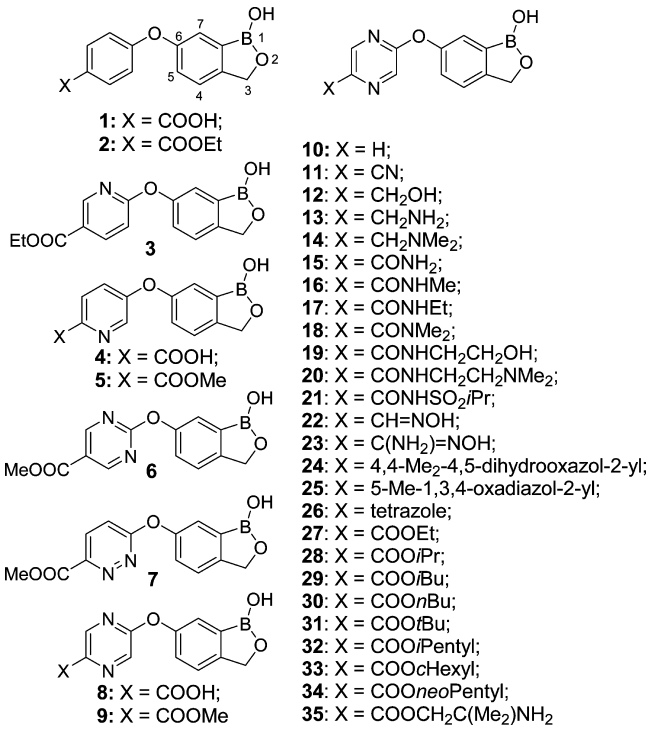


Figure 1. Chemical structures of benzoxaboroles with 6-aryloxy variation (**1**–**9**), substituent changes on the pyrazinyl ring (**10**–**26**), and alterations in the side chain ester group (**27**–**35**).

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.

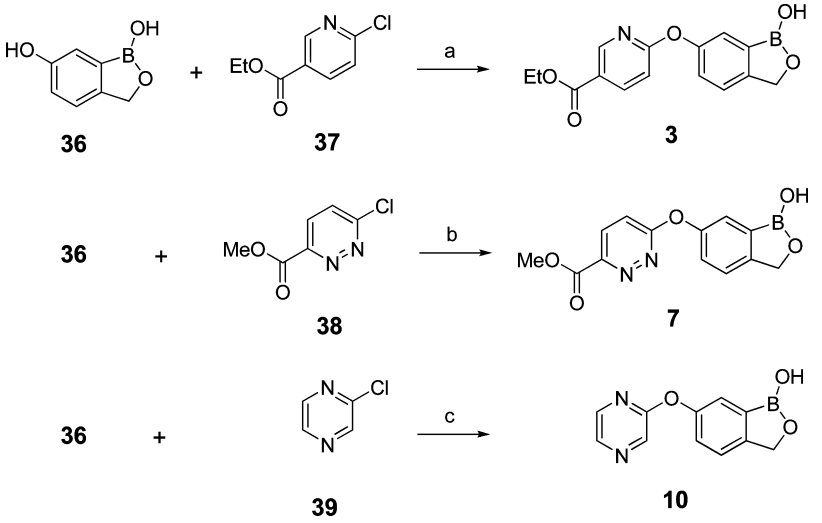
For the synthesis of compound **25**, the chemistry is illustrated in Scheme 7. From a common intermediate **48**, compound **59** was obtained by hydrazine exchange with the ester methoxy group. Further acetylation of **59** gave **60**, which was cyclized to generate **61**. The substitution reaction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

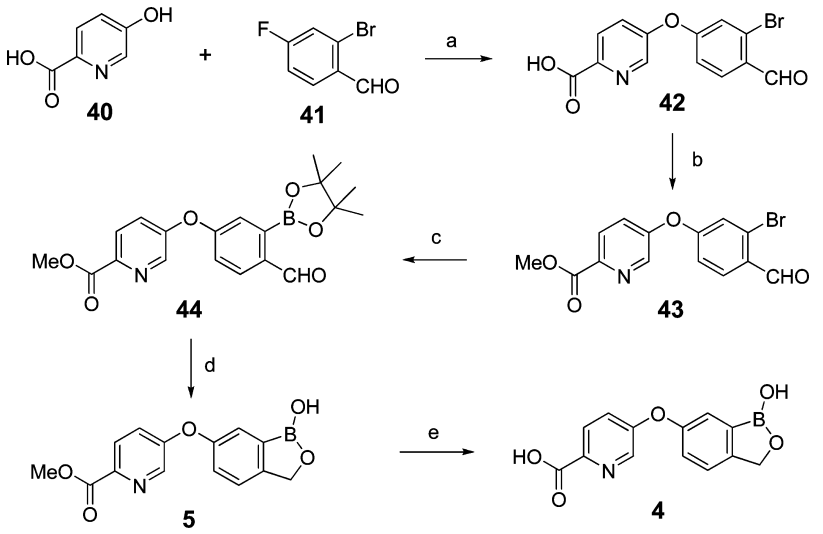
Scheme 1. Synthesis of Compounds 3, 7, and 10[a]



[a]Reagents and conditions: (a) KHMDS, 1,4-dioxane, 0 °C to rt, 15 min, then 80 °C, 22 h, and HCl acidification; (b) Cs_2CO_3, DMF, 50 °C, 1.5 h; (c) Cs_2CO_3, DMF, 75 °C, 0.5 h.

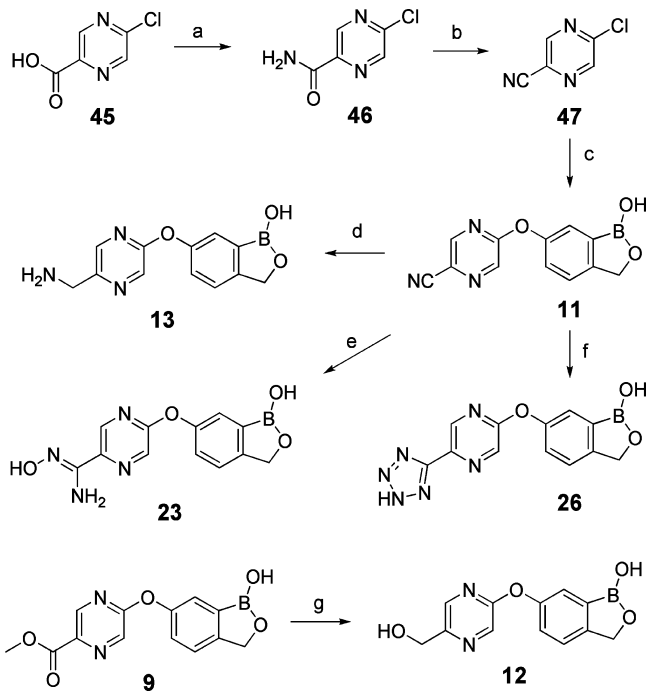
DOI: 10.1021/acs.jmedchem.5b00678
J. Med. Chem. XXXX, XXX, XXX–XXX

Scheme 2. Synthesis of Compounds 4 and 5[a]



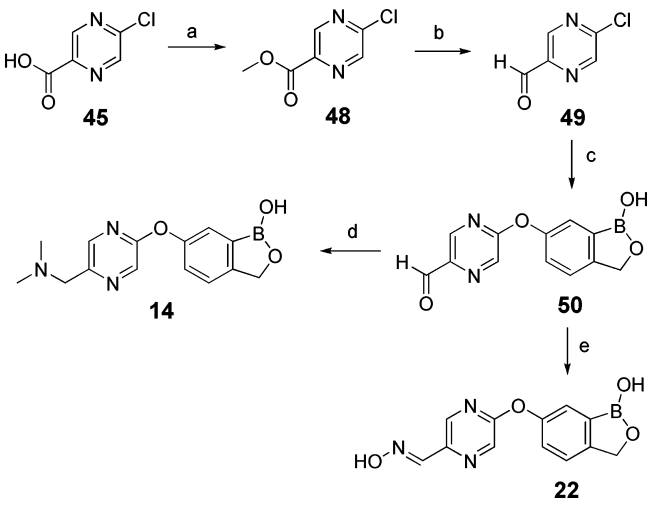
[a]Reagents and conditions: (a) Cs_2CO_3, DMF, 80 °C, 16 h; (b) $SOCl_2$, MeOH, 0–60 °C, 16 h; (c) Pin_2B_2, $Pd(PPh_3)_2Cl_2$, KOAc, 1,4-dioxane, N_2, 100 °C, 0.5 h; (d) $NaBH_4$, MeOH, 0 °C to rt, 1 h, then 1 N HCl; (e) $LiOH·H_2O$, IPA: H_2O (4:1), 40 °C, 1 h, then 1 N HCl.

Scheme 3. Synthesis of Compounds 11–13, 23, and 26[a]



[a]Reagents and conditions: (a) (1) $SOCl_2$, DCM, 50 °C, 16 h, (2) NH_4OH; (b) $(CNCl)_3$ DMF, rt, 1 h; (c) intermediate **36**, Cs_2CO_3, DMF, 50 °C, 2 h; (d) $LiAlH_4$, THF, 0 °C, 50 min; (e) $NH_2OH·HCl$, Na_2CO_3, H_2O, 40 °C, 2 h; (f) NaN_3, NH_4Cl, DMF, rt, 1 h; (g) $LiAlH_4$, THF, 0 °C, 50 min.

Scheme 4. Synthesis of Compounds 14 and 22[a]

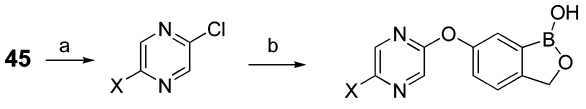


[a]Reagents and conditions: (a) $SOCl_2$, MeOH, 0–60 °C, 16 h; (b) DIBAL-H, THF, −78 °C, 2 h; (c) intermediate **36**, Cs_2CO_3, DMF, 50 °C, 2 h; (d) $Me_2NH·HCl$, $NaBH(OAc)_3$, DCM, rt, 16 h; (e) $NH_2OH·HCl$, NaOH, EtOH, rt, 3 h.

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 IC_{50} 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

DOI: 10.1021/acs.jmedchem.5b00678
J. Med. Chem. XXXX, XXX, XXX–XXX

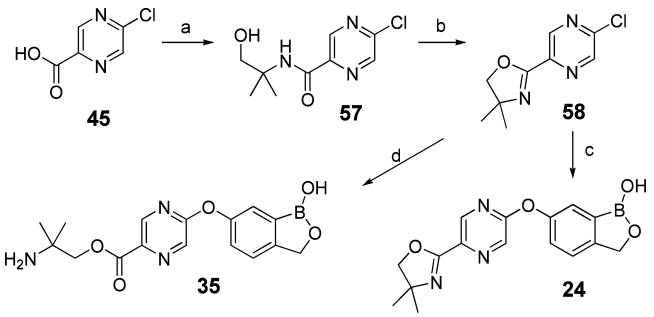
Scheme 5. Synthesis of Compounds 15−21[a]



46: X = $CONH_2$; **15**: X = $CONH_2$;
51: X = CONHMe; **16**: X = CONHMe;
52: X = CONHEt; **17**: X = CONHEt;
53: X = $CONMe_2$; **18**: X = $CONMe_2$;
54: X = $CONHCH_2CH_2OH$; **19**: X = $CONHCH_2CH_2OH$;
55: X = $CONHCH_2CH_2NMe_2$; **20**: X = $CONHCH_2CH_2NMe_2$;
56: X = $CONHSO_2Pr^i$ **21**: X = $CONHSO_2Pr^i$

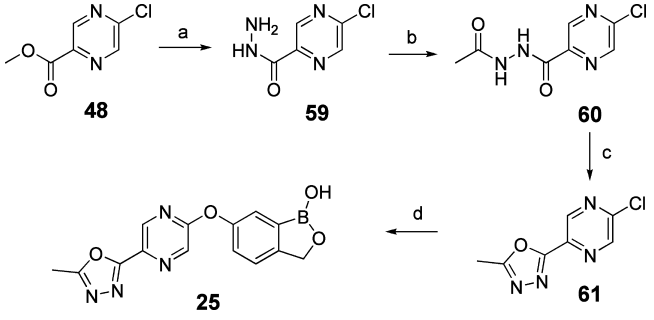
[a]Reagents and conditions: (a) (1) isobutyl chloroformate or $SOCl_2$, (2) NH_4OH for **46**, $MeNH_2$ for **51**, $EtNH_2$ for **52**, Me_2NH for **53**, $NH_2CH_2CH_2OH$ for **54**, $NH_2CH_2CH_2NMe_2$ for **55**, or *i*-$PrSO_2NH_2$ for **56**; (b) for **15**−**20**, intermediate **36**, Cs_2CO_3, DMF, 50 °C, 2 h,; for **21**, intermediate **36**, NaOMe, DMF, 60 °C, 16 h.

Scheme 6. Synthesis of Compounds 24 and 35[a]



[a]Reagents and conditions: (a) HOBT, EDC, 2-amino-2-methyl-1-propanol, TEA, DCM, rt, 30 min; (b) $SOCl_2$, rt, 2 h; (c) intermediate **36**, Cs_2CO_3, DMF, 60 °C, 2 h; (d) intermediate **36**, Cs_2CO_3, DMF, 80 °C, 1 h.

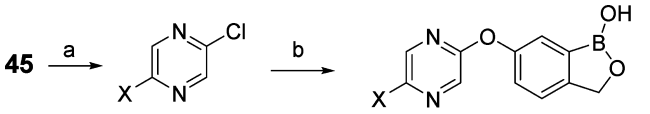
Scheme 7. Synthesis of Compound 25[a]



[a]Reagents and conditions: (a) NH_2NH_2, H_2O, MeOH, 0 °C, 16 h; (b) AcCl, NMM, DCM, rt, 1 h; (c) $POCl_3$, 110 °C, 30 min; (d) intermediate **36**, Cs_2CO_3, DMF, 80 °C, 45 min.

Figure 1, a broad range of structural variation was studied for the left-side group, including hydrogen (**10**), cyano (**11**), hydroxymethyl (**12**), aminomethyl (**13**), dimethylaminomethyl (**14**), carboxamides (**15**−**20**), *N*-sulfonyl carboxamide (**21**), oxime (**22**), carboximidamide (**23**), oxazoline ring (**24**), oxadiazole ring (**25**), and tetrazole (**26**) groups. Activities of these compounds varied widely. Oxazoline and oxadiazole compounds (**24** and **25**) were designed to be bioisosteres of the carboxylic ester group in **9**. Their relatively poor antimalarial activity might indicate that the carboxylic ester

Scheme 8. Syntheses of Ester Compounds 27−34[a]



62: X = COOEt; **27**: X = COOEt;
63: X = COO*i*Pr; **28**: X = COO*i*Pr;
64: X = COO*i*Bu; **29**: X = COO*i*Bu;
65: X = COO*n*Bu; **30**: X = COO*n*Bu;
66: X = COO*t*Bu; **31**: X = COO*t*Bu;
67: X = COO*i*Pentyl; **32**: X = COO*i*Pentyl;
68: X = COO*c*Hexyl; **33**: X = COO*c*Hexyl;
69: X = COO*neo*Pentyl; **34**: X = COO*neo*Pentyl;

[a]Reagents and conditions: (a) (1) $SOCl_2$, 80 °C, 2 h, (2) corresponding alcohol for specific ester intermediates **62**−**69**; 50 °C, 1 h; (b) intermediate **36**, Cs_2CO_3, DMF, 50 °C, 0.5−2 h.

group is 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

DOI: 10.1021/acs.jmedchem.5b00678
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. In Vitro Activity Results for Compounds 1−35 against Cultured *P. falciparum*[a]

entry	cLogD$_{7.4}$[b]	IC$_{50}$ (nM)[a] (W2)	IC$_{50}$ (nM)[a] (3D7)	entry	cLogD$_{7.4}$	IC$_{50}$ (nM) (W2)	IC$_{50}$ (nM) (3D7)
1	−0.37	120 ± 10	1090 ± 170 (72 h)	**20**	−0.53	6660 ± 1350	>5000 ± 0
2	3.60	0%[c]	nt[d]	**21**	−0.25	190 ± 10	130 ± 10
3	2.98	0%[c]	nt[d]	**22**	1.33	1770 ± 470	3000 ± 720
4	−1.64	170 ± 10	540 ± 60	**23**	0.01	>10000 ± 0	>5000 ± 0
5	1.74	23 ± 2	120 ± 0	**24**	1.76	800 ± 70	1460 ± 210
6	1.80	80%[c]	nt[d]	**25**	−0.21	>10000 ± 0	>5000 ± 0
7	1.11	82 ± 16	100 ± 40	**26**	1.31	>10000 ± 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	>10000 ± 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	artemisinin		6 ± 3	na[e]
18	0.72	150 ± 10	960 ± 50	chloroquine		21 ± 6	na[e]
19	−0.27	>10000 ± 0	5900 ± 3200	atovaquone		na[e]	1 ± 0.1

[a]Experimental procedures for the in vitro assays for the W2 and 3D7 strains are described in Supporting Information. IC$_{50}$ 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. [b]cLogD$_{7.4}$ was calculated using ChemAxon software under the condition of pH = 7.4. [c]The value shown is percentage inhibition at 5 μM. [d]Not tested. [e]Not applicable.

Table 2. In Vitro Cytotoxicity Results for Representative Compounds[a]

compd	IC$_{50}$ (μM, Jurkat cell)	compd	IC$_{50}$ (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

[a]The experimental procedure for the in vitro cytotoxicity assay was described previously.[11]

efficacious among the four compounds tested with ED$_{90}$ = 7.0 mg/kg (Table 4). Considering survival of experimental animals, control mice reached lethal parasitemias 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 of cLogD$_{7.4}$ values, respectively, indicating that the carboxylic group is negatively charged (COO^-), 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$_{7.4}$ = 1.13) suggests good cell penetration,[25] which may contribute importantly to its low in vivo ED$_{90}$. 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[a]

	stability in liver microsome				oral PK parameters					intravenous PK parameters			
compd	% remain at 1 h, mouse + NADPH	% remain at 1 h, mouse − NADPH	% remain at 1 h, human + NADPH	% remain at 1 h, human − NADPH	C_{max} (μg/mL)	T_{max} (h)	$AUC_{0\text{-}inf}$ (h·μg/mL)	$T_{1/2}$ (h)	F (%)	CL (mL/h/kg)	V_{ss} (mL/kg)	AUC_{0-inf} (h·μg/mL)	$T_{1/2}$ (h)
8 (an acid)[b]	nd[e]	nd	nd	nd	19.5	0.25	8.62[f]	0.23	67	2326	848	4.3	1.1
9 (an ester)[c]	0	0	0	0	0.16	0.25	0.10	1.0	23	12463	926	0.4	0.3
8 from **9**[d]					0.92	0.25	0.61	1.5	29			0.99	0.13
15 (an amide)[c]	97	100	87	100	1.38	0.25	2.29	2.1	99	2261	973	2.2	0.4

[a]Abbreviations: C_{max}, maximum concentration of drug in plasma; T_{max}, time to maximum concentration of drug in plasma; AUC_{0-inf}, area under the curve extrapolated to infinity; $T_{1/2}$ terminal half-life; F, oral bioavailability; CL, clearance; V_{ss}, volume of distribution at steady state. [b]Oral dosing at 30 mg/kg and intravenous dosing at 10 mg/kg. [c]Oral dosing at 5 mg/kg and intravenous dosing at 5 mg/kg. [d]The acid **8** detected from studies of the ester **9** because there was conversion of **9** to **8** in vivo. [e]Not determined. [f]This is AUC_{0-2h}.

DOI: 10.1021/acs.jmedchem.5b00678
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. Percent Survival of Mice and Efficacies of 4, 8, 9, and 15 in a *P. berghei* Mouse Malaria Model[a]

		percent survival of mice on specific days (%)														
compd	oral dosage	d4[b]	d5	d6	d7	d8	d9	d10	d11	d12	d13	d14	d15	d16-d19	d20	ED_{90}[d] (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	100	60	60	60	0						30.3
	33 mg/kg	100	100	100	100	80	40	40	40	0						
	11 mg/kg	100	100	100	100	60	20	20	20	0						
	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	100	100	100	100	60	40	40	20	0	7.0
	33 mg/kg	100	100	100	100	100	100	100	100	20	0					
	11 mg/kg	100	100	100	100	100	80	80	80	0						
	3 mg/kg	100	100	100	100	80	40	40	40	0						
	1 mg/kg	100	100	100	100	40	0									
15[c]	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	40	0									
	CQ 30 mg/kg	100	100	100	100	100	100	100	100	100	100	100	100	100	20	NA[e]
	vehicle	100	100	40	20	0										NA

[a]Compounds were formulated in a vehicle composed of polyethylene glycol 300, propylene glycol, and water (weight ratio, 55/25/20), and dosed by oral administration once a day for 4 consecutive days. [b]D4 to d20 represent the days of experiment. [c]Compound **15** was tested in a separate 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 control group had 100% survival on day 4 and 0% on day 5. [d]ED_{90}s were based on comparisons of parasitemias with those of mice treated only with vehicle on day 4 after the onset of therapy; [e]Not applicable.

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-oxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles (**9**, **27**–**34**) demonstrated potent in vitro activity, with IC_{50} values of 0.2 to 22 nM against cultured parasites. Compound **9** also showed excellent in vivo efficacy (ED_{90} = 7.0 mg/kg).

EXPERIMENTAL SECTION

General Methods. Starting materials and solvents purchased from chemical companies 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-d_6 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 composed of buffer A (H_2O containing 0.1% phosphoric acid) and buffer B (CH_3CN). The column was eluted with a gradient of 95% buffer A and 5% buffer B to 40% buffer A and 60% 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% O_2, 5% CO_2, and 92% N_2. Parasites synchronized to ring stage by treatments with 5% D-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 NH_4Cl, 0.1% Triton X-100, and 1 nM YOYO-1 in

DOI: 10.1021/acs.jmedchem.5b00678
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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 IC_{50}s were calculated using the Prism 3.0 program (GRAPHPAD Software). Goodness of fit was assessed by R^2 values, and meaningful dose−response curves yielded R^2 values >0.95.

Inhibition of 3D7 strain parasites was assessed using the [^{3}H]-hypoxanthine incorporation assay.[26] 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-sucrose (Sigma-Aldrich), 0.3% glutamine (Sigma-Aldrich), and 5 mM hypoxanthine (Sigma-Aldrich). Cultures were maintained at 37 °C at an atmosphere of 5% O_2, 5% CO_2, and 95% N_2. 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, [^{3}H]-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 no. 1450-441) to determine incorporation of [^{3}H]-hypoxanthine. Radioactivity was measured using a microbeta counter (PerkinElmer). Data are normalized using the incorporation of the positive control (infected erythrocytes without drug). IC_{50} values were determined using Excel and Grafit 5 software. Values were determined from at least three independent experiments, and standard deviations were calculated.

Method for Testing in Vitro 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 by spiking hepatocytes with 10 mM DMSO 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 **8**, the doses were 10 mg/kg for iv and 30 mg/kg for oral, and the formulation used 100% saline. For benzoxaborole **9**, 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 **15**, 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 6×10^6 *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 Giemsa stained smears. All counts within a single experiment were performed by the same investigator. The ED_{90} 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 ED_{90} 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, 24 mmol, 3 equiv) at 0 °C. The reaction was stirred at room temperature (rt) for 15 min, and then intermediate **37** (2.97g, 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, combined, washed with brine, dried over anhydrous Na_2SO_4, 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. ^{1}H NMR (300 MHz, DMSO-d_6): 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, ESI+).

Methyl 5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)picolinate (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 Cs_2CO_3 (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 $MgSO_4$, filtered, and evaporated to a solid (6 g). 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 $SOCl_2$ (3.6 mL, 48.90 mmol, 2.5 equiv) under N_2. Then the reaction was heated to 60 °C and stirred overnight. The mixture was evaporated to a residue, which was poured into $NaHCO_3$ (50 mL) and extracted with EA (3 × 20 mL). The combined organic phases was washed with brine, dried over Na_2SO_4, filtered, and evaporated to **43** as solid (3.8 g, 93% yield). To a solution of **43** (0.8 g, 2.38 mmol), Pin_2B_2 (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 $Pd(PPh_3)_2Cl_2$ (170 mg, 0.24 mmol, 0.1 equiv) under N_2. 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 Na_2SO_4, 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 $NaBH_4$ (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 Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography to the desired product **5** as white solid (0.2 g, 71% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.26 (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

DOI: 10.1021/acs.jmedchem.5b00678
J. Med. Chem. XXXX, XXX, XXX−XXX

220 nm and 99.3% at 254 nm. Mass: m/z = 286.1 (M + 1, ESI+). To a solution of **5** (0.3 g, 1.05 mmol) in 2-propanol (8.9 mL) was added $LiOH·H_2O$ (88 mg, 2.10 mmol, 2 equiv) in H_2O (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 Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography to the desired product **4** as white solid (0.2 g, 74% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.00 (br s, 1H), 9.37 (s, 1H), 8.44 (s, 1H), 8.02 (d, 1H), 7.52−7.28 (m, 4H), 4.99 (s, 2H) ppm. HPLC area: 100% at 220 nm and 100% at 254 nm. Mass: m/z = 272 (M + 1, ESI+).

Methyl 6-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyridazine-3-carboxylate (7). To a solution of **38** (172 mg, 1 mmol, 1 equiv) in DMF (4 mL) were added **36** (150 mg, 1 mmol, 1 equiv) and Cs_2CO_3 (813 mg, 2.5 mmol, 2.5 equiv) at rt. The reaction was stirred at 50 °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 $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography to the desired product **7** (40 mg, 14% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.30 (s, 1H), 8.25 (d, 1H), 7.51−7.61 (m, 3H), 7.39 (d, 1H), 5.04 (s, 2H), 3.93 (s, 3H) ppm. HPLC area: 96.1% at 220 nm and 96.5% at 254 nm. Mass: m/z = 287 (M + 1, ESI+).

6-(Pyrazin-2-yloxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol (10). To a solution of **39** (0.3 g, 2.8 mmol, 1.5 equiv) in DMF (10 mL) were added Cs_2CO_3 (1.5 g, 4.75 mmol, 2.5 equiv) and **36** (0.28 g, 1.9 mmol). The reaction mixture was stirred at 75 °C for 0.5 h. After completion, the reaction was diluted with H_2O (20 mL) and extracted with EA (2 × 10 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous Na_2SO_4, and filtered. The residue after rotary evaporation was purified by column chromatography to the desired product **10** (50 mg, 8% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.57 (d, 1H), 8.37 (d, 1H), 8.19 (dd, 1H), 7.49−7.47 (m, 2H), 7.31 (dd, 1H), 5.01 (s, 2H) ppm. HPLC area: 96.5% at 220 nm and 95.9% at 254 nm. Mass: m/z = 229 (M + H, ESI+).

5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)-pyrazine-2-carbonitrile (11). To a solution of **45** (1.0 g, 6.3 mmol, 1 equiv) in DCM (31 mL) was added $SOCl_2$ (31 mL) at rt. The reaction was heated to 50 °C overnight and then concentrated. The residue after rotary evaporation was mixed with $NH_3·H_2O$ and then extracted with DCM (20 mL). The organic phase was washed with saturated Na_2CO_3 (50 mL) and H_2O (50 mL), dried over anhydrous $MgSO_4$, and filtered. The residue after rotary evaporation was purified by column chromatography to the amide compound **46** (0.6 g, 67% yield). To a solution of **46** (0.6 g, 3.80 mmol) in DMF (10 mL) was added $(CNCl)_3$ (0.84g, 4.56 mmoL, 1.2 equiv) at rt. The mixture was stirred at rt for 1 h before H_2O (100 mL) was added. The mixture was extracted with EA (3 × 40 mL). The organic phases were combined, washed with 0.5 N HCl (2 × 50 mL), saturated Na_2CO_3 (2 × 50 mL), and brine (300 mL), dried over anhydrous $MgSO_4$, and filtered. The residue after rotary evaporation was purified by column chromatography to **47** (0.3 g, 60% yield). To a solution of **47** (110 mg, 0.80 mmol, 1.2 equiv) in DMF (2.2 mL) were added **36** (100 mg, 0.67 mmol) and Cs_2CO_3 (0.54g, 1.68 mmol, 2.5 equiv) in one portion at rt. The reaction mixture was stirred at 50 °C for 2 h, poured into 2 N HCl (100 mL), and then extracted with EA (3 × 50 mL). The organic phases were combined, washed with brine, dried over $MgSO_4$, filtered, and evaporated to a solid (150 mg). The residue was purified by column chromatography to the desired product **11** as a white solid (40 mg, 25% yield). ^{1}HNMR (300 MHz, DMSO-d_6): δ 9.26 (s, 1H), 8.76 (s, 1H), 8.74 (s, 1H), 7.51−7.48 (m, 2H), 7.36 (dd, 1H), 5.01 (s, 2H) ppm. HPLC area: 99.2% at 220 nm and 98.6% at 254 nm. Mass: m/z = 505 (2M − 1, ESI−).

6-(5-(Hydroxymethyl)pyrazin-2-yloxy)benzo[*c*][1,2]-oxaborol-1(3*H*)-ol (12). To a suspension of $LiAlH_4$ (342 mg, 9 mmol, 3 equiv) in THF (10 mL) was added dropwise a solution of **9** (860 mg, 3.0 mmol) in THF (5 mL). The reaction mixture was stirred at 0 °C for 50 min, diluted with H_2O (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 Na_2SO_4, and filtered. The residue after rotary evaporation was purified by column chromatography to the product **12** (210 mg, 27% yield). ^{1}H NMR (300 MHz, DMSO-d_6): 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 +).

6-(5-(Aminomethyl)pyrazin-2-yloxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (13). To a solution of $LiAlH_4$ (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 H_2O (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/MeOH and then concentrated to the desired solid product **13** (22 mg, 27% yield). ^{1}H NMR (300 MHz, DMSO-d_6): 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+).

6-(5-((Dimethylamino)methyl)pyrazin-2-yloxy)benzo[*c*][1,2]-oxaborol-1(3*H*)-ol (14). A solution of **45** (1.58g, 10 mmol) in $SOCl_2$ (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 H_2O (30 mL) and extracted with EA (2 × 10 mL). The organic phases were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (EA:PE = 10%) to the methyl ester **48** (1.4 g, 81% yield). To a solution of **48** (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 × 100 mL). The organic phases were combined, washed with brine, dried over $MgSO_4$, 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 Cs_2CO_3 (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 with brine, dried over $MgSO_4$, filtered, and evaporated. Then the residue was 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 1.5 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). ^{1}H NMR (300 MHz, DMSO-d_6): 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), 3.72 (s, 2H), 2.31 (s, 6H) ppm. HPLC area: 98.1% at 220 nm and 97% at 254 nm. Mass: m/z = 286 (M + H, ESI+).

5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)-pyrazine-2-carboxamide (15). A solution of **46** (516 mg, 3 mmol), **36** (450 mg, 3 mmol, 1 equiv), and Cs_2CO_3 (2.46g, 7.5 mmol, 2.5 equiv) in DMF (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 $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography to the desired product **15** (460 mg, 56% yield) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.70 (s, 1H), 8.57 (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 + 1, ESI+).

5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)-*N*-methylpyrazine-2-carboxamide (16). This compound was prepared by the similar method described above for the synthesis of **15**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.72−8.70 (m, 1H),

DOI: 10.1021/acs.jmedchem.5b00678
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8.68 (s, 1H), 8.58 (s, 1H), 7.52−7.49 (m, 2H), 7.36 (dd, 1H), 5.03 (s, 2H), 2.82 (d, 3H) ppm. HPLC area: 100.0% at 220 nm and 96.5% at 254 nm. Mass: m/z = 286 (M + H, ESI+) and 308 (M + Na, ESI+).

***N*-Ethyl-5-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxamide (17).** This compound was prepared by the similar method described above for the synthesis of **15**. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.25 (s, 1H), 8.77 (t, 1H), 8.68 (s, 1H), 8.58 (s, 1H), 7.52−7.49 (m, 2H), 7.35 (dd, 1H), 5.03 (s, 2H), 3.37−3.27 (m, 2H), 1.15 (t, 3H) ppm. HPLC area: 99% at 220 nm and 99.3% at 254 nm. Mass: m/z = 300 (M + H, ESI+).

5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)-*N*,*N*-dimethylpyrazine-2-carboxamide (18). This compound was prepared by the similar method described above for the synthesis of **15**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.23 (s, 1H), 8.54 (s, 1H), 8.38 (s, 1H), 7.51−7.49 (m, 2H), 7.35 (dd, 1H), 5.02 (s, 2H), 3.02 (s, 6H) ppm. HPLC area: 99.6% at 220 nm and 99.2% at 254 nm. Mass: m/z = 300 (M + 1).

5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)-*N*-(2-hydroxyethyl)pyrazine-2-carboxamide (19). This compound was prepared by the similar method described above for the synthesis of **15**. ^{1}H NMR (300 MHz, DMSO-d_6): 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), 4.82 (t, 1H), 3.55−3.49 (m, 2H), 3.40−3.35 (m, 2H) ppm. HPLC area: 97.8% at 220 nm and 99.0% at 254 nm. Mass: 316 (M + 1, ESI +).

***N*-(2-(Dimethylamino)ethyl)-5-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxamide hydrochloride (20).** This compound was prepared by the similar method described above for the synthesis of **15** and was converted to HCl salt. ^{1}H NMR (300 MHz, DMSO-d_6): 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), 3.28−3.32 (m, 2H), 2.82 (s, 6H) ppm. HPLC area: 99.5% at 220 nm and 99.6% at 254 nm. Mass: m/z = 343 (M + 1, ESI+).

5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)-*N*-(isopropylsulfonyl)pyrazine-2-carboxamide (21). This compound was prepared by the similar method described above for the synthesis of **15**. ^{1}H NMR (300 MHz, DMSO-d_6): 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 97.9% at 254 nm. Mass: m/z = 378 (M + 1, ESI +).

(*E*)-5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carbaldehyde oxime (22). To a solution of **50** (100 mg, 0.39 mmol) and hydroxylamine hydrochloride (31 mg, 0.45 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 water (5 mL), and extracted with EA (2 × 10 mL). The organic phases were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (MeOH:DCM = 1%) to **22** (38 mg, 36% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.77 (s, 1H), 9.25 (s, 1H), 8.56 (s, 1H), 8.52 (s, 1H), 8.14 (s, 1H), 7.50−7.47(m, 2H), 7.35 (d, 1H), 5.02 (s, 2H) ppm. HPLC area: 94.6% at 220 nm and 98.8% at 254 nm.

(*Z*)-*N*′-Hydroxy-5-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]-oxaborole-6-yloxy)pyrazine-2-carboximidamide (23). To a solution of **11** (250 mg, 0.99 mmol) and hydroxylamine hydrochloride (137 mg, 1.98 mmol, 2.0 equiv) in H_2O (3 mL) was added a solution of Na_2CO_3 (209 mg, 19.8 mmol, 2.0 equiv) in H_2O (0.9 mL). The reaction mixture was stirred at 40 °C for 3 h, poured into water (20 mL), and extracted with EA (4 × 15 mL). The organic phases were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (MeOH:DCM = 1%) to the product **23** (34 mg, 12% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.99 (s, 1H), 9.23 (s, 1H), 8.59 (s, 1H), 8.52 (s, 1H), 7.52−7.43 (m, 2H), 7.34 (dd, 1H), 5.88 (s, 2H), 5.02 (s, 2H) ppm. HPLC area: 100% at 220 nm and 98.2% at 254 nm. Mass: m/z = 287 (M + H, ESI+).

6-(5-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)pyrazin-2-yloxy)-benzo[*c*][1,2]oxaborol-1(3*H*)-ol (24). To a solution of **45** (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 DCM (40 mL, c = 0.1) was added TEA (1.2 g, 12 mmol, 3 equiv). The reaction was stirred at rt for 30 min, and a normal workup resulted in the desired product **57** (670 mg, 80% yield) as yellow oil. To $SOCl_2$ (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 $NaHCO_3$, and then separated. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography to the desired product **58** (180 mg, 50% yield) as a white solid. To a solution of **58** (85 mg, 0.4 mmol) in DMF (4 mL) were added **36** (60 mg, 0.4 mmol, 1 equiv) and Cs_2CO_3 (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 $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography to the desired product **24** (12 mg, 12% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (s, 1H), 8.65 (s, 1H), 8.62 (s, 1H), 7.48−7.51 (m, 2H), 7.35 (dd, 1H), 5.02 (s, 2H), 4.14 (s, 2H), 1.30 (s, 6H) ppm. HPLC area: 95.3% at 220 nm and 94.9% at 254 nm. Mass: m/z = 326 (M + H, ESI+).

6-(5-(5-Methyl-1,3,4-oxadiazol-2-yl)pyrazin-2-yloxy)benzo-[*c*][1,2]oxaborol-1(3*H*)-ol (25). To a solution of **48** (172 mg, 1 mmol) in MeOH (7 mL) was added a solution of hydrazine hydrate (0.11 mL, 2 mmol, 2 equiv) in MeOH dropwise at 0 °C. The reaction mixture was stirred for 16 h, filtered, and concentrated to the desired product **59** (100 mg, 58% yield). To a solution of **59** (100 mg, 0.58 mmol) and NMM (203 mg, 1.74 mmol, 3 equiv) 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 H_2O (10 mL), and extracted with DCM (20 mL). The organic phase was washed with 1 N HCl (10 mL), 1N $NaHCO_3$ (15 mL), and brine (15 mL), dried over anhydrous Na_2SO_4, 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, 0.42 mmol, 1 equiv) in $POCl_3$ (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 1N $NaHCO_3$ (10 mL), and extracted with EA (15 mL). The organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, 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, 0.306 mmol) in DMF (1 mL) were added Cs_2CO_3 (251 mg, 0.765 mmol, 2.5 equiv) and **36** (46 mg, 0.306 mmol, 1 equiv). The reaction mixture was stirred at 80 °C for 45 min, diluted with H_2O (10 mL), and extracted with EA (10 mL). The organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The residue after rotary evaporation was purified by column chromatography to the desired product **25** (35 mg, 50% yield). ^{1}H NMR (300 MHz, DMSO-d_6): 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).

6-(5-(1*H*-Tetrazol-5-yl)pyrazin-2-yloxy)benzo[*c*][1,2]-oxaborol-1(3*H*)-ol (26). To a solution of **11** (200 mg, 0.75 mmol) in DMF (2 mL) were added NH_4Cl (68 mg, 1.27 mmol, 1.7 equiv) and NaN_3 (82 mg, 1.27 mmol, 1.7 equiv). The reaction mixture was stirred at 95 °C overnight, diluted with H_2O (40 mL), and extracted with EA (2 × 20 mL). The aqueous phase was adjusted to pH < 7 with 0.5 N HCl and extracted with EA (2 × 30 mL). The combined organic phases were washed with brine (2 × 30 mL), dried over anhydrous Na_2SO_4, and filtered. The residue after rotary evaporation was purified by column chromatography to the desired product **26** (40 mg, 18% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.32 (s, 1H), 9.06 (s, 1H), 7.18 (d, 1H), 7.09 (s, 1H), 6.87 (dd, 1H), 4.86 (s, 2H) ppm. HPLC area: 99.8% at 220 nm and 93.9% at 254 nm. Mass: m/z = 319 (M + Na, ESI+).

Ethyl 5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (27). A solution of **45** (2g, 12.62

DOI: 10.1021/acs.jmedchem.5b00678
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mmol) in $SOCl_2$ (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 H_2O (40 mL), and extracted with EA (3 × 50 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, 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 Cs_2CO_3 (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 $MgSO_4$, 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-d_6): 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, ESI+).

Isopropyl 5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (28). This compound was prepared by following the methodology similar to that described above for **27**. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.23 (s, 1H), 8.74 (s, 1H), 8.65 (s, 1H), 7.53−7.50 (m, 2H), 7.36 (dd, 1H), 5.21−5.16 (septet, 1H), 5.03 (s, 2H), 1.33 (d, 6H) ppm. HPLC area: 97.4% at 220 nm and 98.7% at 254 nm. Mass: m/z = 315 (M + H, ESI+).

Isobutyl 5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (29). This compound was prepared by following the methodology similar to that described above for **27**. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.77 (s, 1H), 8.68 (s, 1H), 7.54−7.50 (m, 2H), 7.36 (dd, 1H), 5.03 (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, ESI+).

***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 (300 MHz, DMSO-d_6): 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, ESI+).

***tert*-Butyl 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 (300 MHz, DMSO-d_6): δ 9.26 (s, 1H), 8.71 (s, 1H), 8.64 (s, 1H), 7.51−7.49 (m, 2H), 7.35 (dd, 1H), 5.02 (s, 2H), 1.55 (s, 9H) ppm. HPLC area: 98.8% at 220 nm and 99.0% at 254 nm. Mass: m/z = 351 (M + Na, ESI+).

Isopentyl 5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (32). This compound was prepared by following the methodology similar to that described above for **27**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.74 (s, 1H), 8.66 (s, 1H), 7.51−7.49 (m, 2H), 7.34 (dd, 1H), 5.02 (s, 2H), 4.33 (t, 2H), 1.78−1.67 (m, 1H), 1.65−1.56 (m, 2H), 0.90 (d, 6H) ppm. HPLC area: 98.6% at 220 nm and 98.5% at 254 nm. Mass: m/z = 365 (M + Na, ESI+).

Cyclohexyl 5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (33). This compound was prepared by following the methodology similar to that described above for **27**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.26 (s, 1H), 8.76 (s, 1H), 8.66 (s, 1H), 7.53−7.49 (m, 2H), 7.36 (dd, 1H), 5.03 (s, 2H), 4.97−4.90 (m, 1H), 1.88−1.23 (m, 10H) ppm. HPLC area: 95.1% at 220 nm and 95.1% at 254 nm. Mass: m/z = 377 (M + Na).

Neopentyl 5-(1-Hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (34). This compound was prepared by following the methodology similar to that described above for **27**. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.29 (s, 1H), 8.84 (s, 1H), 8.75 (s, 1H), 7.59−7.55 (m, 2H), 7.43 (dd, 1H), 5.08 (s, 2H), 4.09 (s, 2H), 1.04 (s, 9H) ppm. HPLC area: 96.1% at 220 nm and 98.1% at 254 nm. Mass: m/z = 365 (M + Na, ESI+).

2-Amino-2-methylpropyl 5-(1-Hydroxy-1,3-dihydrobenzo-[*c*][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate Hydrochloride (35). To a solution of **58** (85 mg, 0.4 mmol, 1 equiv) in DMF (2 mL) was added **36** (60 mg, 0.4 mmol) and Cs_2CO_3 (325 mg, 1 mmol, 2.5 equiv) at rt. The mixture was stirred at 80 °C for 1 h, poured into 1 N HCl (20 mL) and concentrated directly to a residue. The residue was purified by column chromatography to give **35** (17 mg, 17% yield) as a light-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): 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, ESI+).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures illustrating mice survival and efficacy ED_{90} 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.

■ ACKNOWLEDGMENTS

We thank Medicines for Malaria Venture (MMV) and the National Institutes of Health (AI095324) for financial support. We also thank Dr. David Sullivan for calculating $cLogD_{7.4}$ values of the compounds.

■ ABBREVIATIONS USED

NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography; ESI, electrospray ionization; DMSO, dimethyl sulfoxide; KHMDS, potassium hexamethyldisilazide; EA, ethyl acetate; DCM, dichloromethane; TLC, thin layer chromatography; PE, petroleum ether; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; m-CPBA, *meta*-chloroperbenzoic acid; HOBT, hydroxybenzotriazole; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; NMM, *N*-methylmorpholine

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DOI: 10.1021/acs.jmedchem.5b00678
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DOI: 10.1021/acs.jmedchem.5b00678
J. Med. Chem. XXXX, XXX, XXX−XXX