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Article

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Benzoxaborole Antimalarial Agents. Part 5. Lead Optimization of Novel Amide Pyrazinyloxy Benzoxaboroles and Identification of a Preclinical Candidate

Yong-Kang Zhang,[*,†] *Jacob J. Plattner,*[†] *Eric E. Easom,*[†] *Robert T. Jacobs,*[†] *Denghui Guo,*[§] *Yvonne R. Freund,*[†] *Pamela Berry,*[†] *Vic Ciaravino,*[†] *John C. L. Erve,*[†] *Philip J. Rosenthal,*[§] *Brice Campo,*[‡] *Francisco-Javier Gamo,*[‖] *Laura M. Sanz,*[‖] *Jianxin Cao*[⊥]

[†]Anacor Pharmaceuticals, Inc., 1020 E. Meadow Circle, Palo Alto, CA 94303, USA

[§]Department of Medicine, University of California, San Francisco, Box 0811, San Francisco, California 94143, USA

[‡]Medicines for Malaria Venture, International Center Cointrin, Block G, 20 route de Pré-Bois, POB 1826, CH - 1215 Geneva

[‖]Diseases of the Developing World, GlaxoSmithKline, Severo Ochoa 2, 28760, Tres Cantos, Spain

[⊥]Shanghai ChemPartner, 998 Ha-lei Road, Zhangjiang High-tech Park, Pudong New Area, Shanghai 201203, China

KEYWORDS: Benzoxaborole; Antimalarial agent; Structure–activity relationship; Malaria; *Plasmodium falciparum*; *In vivo* efficacy

ABSTRACT: Carboxamide pyrazinyloxy benzoxaboroles were investigated with the goal to identify a molecule with satisfactory antimalarial activity, physicochemical properties, pharmacokinetic profile, *in vivo* efficacy, and safety profile. This optimization effort discovered **46**, which met our target candidate profile. Compound **46** had excellent activity against cultured *Plasmodium falciparum*, and *in vivo* against *P. falciparum* and *P. berghei* in infected mice. It exhibited good PK properties in mouse, rat and dog. It was highly active against other eleven *P.*

falciparum strains, which are mostly resistant to chloroquine and pyrimethamine. The rapid parasite *in vitro* reduction and *in vivo* parasite clearance profile of **46** were similar to those of artemisinin and chloroquine, two rapid-acting antimalarials. It was non-genotoxic in an Ames assay, an *in vitro* micronucleus assay and an *in vivo* rat micronucleus assay when dosed orally up to 2000 mg/kg. The combined properties of this novel benzoxaborole support its progression to preclinical development.

INTRODUCTION

Malaria, a parasitic infection by the *Plasmodium* genera and spread through the bites of infected mosquitoes, was responsible for an estimated 212 million clinical cases and 429,000 deaths worldwide in 2015, mostly among children under the age of five.[1] This terrible disease, in particular infection with *Plasmodium falciparum*, the most virulent human malaria parasite, traps communities into poverty and disproportionately affects poor people who have limited access to health care. Following the discovery of artemisinin in the 1970's [2-7], semi-synthetic artemisinin-based combination therapies (ACTs) have become frontline medicines to treat malaria.[1] However, *Plasmodium falciparum* parasites in southeast Asia have shown evidence of resistance to artemisinins. [8-10] A deterioration of the susceptibility of *P. falciparum* to artemisinins and the possibility that resistance will spread beyond the Greater Mekong sub-region are threats to global strategies for the control, elimination and eradication of malaria. In addition, resistance to available artemisinin partner drugs is already widespread.[9] Therefore, there is an urgent need to enhance the pipeline of antimalarial medications that counter resistance and that are safe and easy for use in the most vulnerable populations.[11-15] In the process of creating richer antimalarial development pipelines, many new chemical entities with different structures and sometimes also with new mechanisms of action have entered preclinical and clinical development for the

potential treatment of malaria in the last decade.[16-18] Maintaining such momentum as a sustained effort is very important to the future of antimalarial therapeutics because attrition is expected during drug development, and only a small portion of development compounds may reach the market.[19] However, antimalarial drug discovery presents unique challenges. New antimalarials should ideally meet multiple criteria, including excellent potency, ideally against multiple plasmodial species; oral bioavailability, ideally with cure after a single dose; safety in children and pregnant women; and low cost of production.[18]

We recently reported that 6-(2-(methoxycarbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (**1** in Fig. 1) demonstrated potent *in vitro* activity against cultured parasites (IC_{50} = 1.4 nM and 1.9 nM against *P. falciparum* W2 and 3D7 strains, respectively) and excellent *in vivo* efficacy against *P. berghei* in infected mice (ED_{90} = 7.0 mg/kg).[20] The limitation of this molecule was its poor oral pharmacokinetic (PK) profile, exemplified by a short half-life ($t_{1/2}$ = 1 h) and low bioavailability (F = 23%) in mice. This profile is readily attributable to hydrolysis of the methyl ester to the corresponding carboxylic acid. During this phase of the project, it was also found that the primary carboxamide analog (**2** in Fig. 1) had a much-improved PK profile in mice ($t_{1/2}$ = 2.1 h and F = 99%) and moderate *in vivo* efficacy (ED_{90} = 38.7 mg/kg) despite being significantly less potent *in vitro* (IC_{50} = 210 nM against W2 and 350 nM against 3D7).[20] We also learned that incorporation of a 7-Me substituent on the benzoxaborole core can improve PK properties. Therefore, we focused our effort on the 7-methyl benzoxaborole pyrazine carboxamide scaffold to optimize *in vitro* potency, stability of the carboxamide group and PK profile in order to ultimately provide molecule(s) with better *in vivo* efficacy. More specifically, we report here systematic modification of carboxamide region to generate analogs **3**-**51** (Fig. 1), which were evaluated against *P. falciparum*; selected compounds

were further profiled in a range of *in vitro* ADME, *in vivo* PK and *in vivo* mouse efficacy models. The optimized lead molecule, 6-(2-((3-hydroxy-3-methylazetidin-1-yl)carbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-7-methyl-2,1-benzoxaborole (**46**), was progressed to evaluate potency against resistant *P. falciparum* strains, *in vivo* parasite reduction rate, and to preliminary genotoxicity studies. The results obtained on **46** support its progression to preclinical development.

CHEMISTRY

The chemistry for the synthesis of compounds **1** and **2** was described previously.[20] Preparation of molecules **3**-**51** shared a common benzoxaborole intermediate **58**, for which the synthetic route is illustrated in Scheme 1. Formylation of 2-methylresorcinol (**52)** generated the aldehyde **53**, which was selectively protected to give **54**. The remaining hydroxyl group of **54** was converted to a boronate ester in **56** via the triflate **55** and palladium-mediated boronylation. Reduction of the aldehyde **56** and simultaneous cyclization produced the 6-O-benzyl benzoxaborole **57**, which was deprotected by catalytic hydrogenation to give **58**. Methods for the synthesis of various substituted 2-aminoethanol intermediates are illustrated in Schemes 2-5. The condensation product **63** from **61** and **62** reacted with the Grignard reagent **60**, which was generated from **59**, to provide the silylmethyl adduct **64**. Oxidative desilylation of **64** gave **65**, followed by hydrolysis to yield the desired 2-aminoethanol **66**. Its enantiomer **68** was obtained by following the same methods starting from **67**. For the preparation of **74** in Scheme 3, the triflate (**70**) of trifluoroethanol (**69)** was used to alkylate **71,** providing the alkylation product **72** that was hydrolyzed to provide the amino acid **73**, which was reduced to the aminoalcohol **74**. In Scheme 4, the ester group of the cyclization product **77** from **75** and **76** was reduced to the alcohol **78**. Methylation of **78** produced the ether **79**, which was hydrolyzed to give the 2-

aminoethanol **80** containing the ether side group. For the synthesis of the thioether analog **86**, the Boc-protected ester product **83** from **81** and **82** was methylated to give **84** that was hydrolyzed under an acidic condition to afford **85**, followed by reduction resulting in **86**. As shown in Scheme 5, the tertiary alcohols **93-95** and **102-104** can be conveniently generated by Grignard reaction from their corresponding amino acid methyl esters **90-92** and **99-101**, which were obtained from amino acids **87-89** and **96-98**, respectively. Synthesis of substituted azetidines **112-117** is shown in Scheme 6. Protected azetidinone **105** was reacted with nucleophilic agents, RMgBr or Me_3SiCF_3, to give the alcohols **106-111** that were deprotected by catalytic hydrogenation to provide the hydroxylated azetidines **112-117**. With both the key benzoxaborole and amine fragments in hand, two general methods to generate the amides **3-51** have been used as illustrated in Scheme 7. In general method A, the pyrazine amides were prepared from amines **118** and acid **119** prior to the substitution reaction between the phenolic benzoxaborole **58** and the amide pyrazinyl chloride **120**. In general method B, the amide formation was conducted on the acid intermediate **123** derived from the ester **122**, which was produced by a substitution reaction between **58** and **121**.

RESULTS AND DISCUSSION

Structure-Activity Relationships. The *in vitro* activities (IC_{50}s) of test compounds for growth inhibition of the 3D7 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *P. falciparum* were determined (see Table 1). Compound **3** with a *N*-(2'-methoxyethyl) group at the amide had similar potency as **4** with a *N*-(2'-hydroxyethyl) group. Compound **5** with an extra 2'-methyl, designed to decrease oxidative metabolism of the hydroxyl group, was slightly less potent than **4**, whereas the 2',2'-dimethyl analog **6** was equipotent with **4**. The corresponding 1',1'-dimethyl analog **7** was also equipotent with **4**, but its cyclopropyl analog **8** had decreased

activity. More analogs (**9**-**29**) with various 1'-monoalkyl substituents in the amide side chain, to modulate lipophilicities and improve PK properties, had potencies ranging from 31 to 1990 nM and showed that the (*R*)-enantiomer was generally slightly more active than the (*S*)-isomer. Compounds with 1',2'-dialkyls (**30**-**33**) mostly gave potency around 200 nM. Similar activities were seen with (**34**-**39**) that have 1',2',2'-trialkyls in the amide side chain. Targeting to improve the metabolic stability of the amide bond, *N*,*N*-disubstituted amide analogs (**40**-**44**) were synthesized, and their IC_{50} values ranged from 48 to 437 nM indicating that these substitutions were tolerated. Considering this *N*,*N*-disubstitution pattern and a previous observation that compounds **37** and **38** containing a terminal tertiary alcohol had good activity promoted the idea to synthesize the cyclic pyrrolidinyl molecule **45**, which showed IC_{50} = 139 nM (W2 strain). Further structural simplification to remove the chiral center in **45** resulted in the methylazetidinyl compound **46** with improved potency of IC_{50} 33 nM (W2 strain) and 43 nM (3D7 strain). Five additional azetidinyl analogs (**47**-**51**) with ethyl, n-propyl, isopropyl, cyclopropyl or trifluoromethyl replacing the 3'-methyl at azetidine in **46** were also very active (IC_{50} from 7 nM to 134 nM).

Metabolic Stability and Pharmacokinetic Study. As *in vitro* potency was maintained with a relatively broad array of amide substitution patterns, a key driver for the project was to build good pharmacokinetic properties into the series to enable provision of a single dose cure. This feature is critical for treatment of malaria patients in disease endemic areas to ensure compliance. Therefore, the pharmacokinetic properties of selected compounds following oral administration to mice were determined (Table 2). The hydroxyethyl amide **9** had high plasma concentration, good exposure, moderate half-life, and excellent bioavailability. It was hypothesized that increasing length of exposure (as indicated by half-life) was important to achieve our goal of a

single dose cure molecule. In order to achieve this goal, we further hypothesized that increasing molecule lipophilicity would lead to a higher volume of distribution and a longer half-life. Of course, it was important that changes in lipophilicity could not be large, as it was expected that metabolic stability could be adversely affected by such changes. Therefore, compounds **9** with methyl substitution on the amide side chain (cLogD = 0.66), **20** with trifluoroethyl (cLogD = 1.54) and **14** with *n*-propyl (cLogD = 1.53) were compared. Compound **14** gave a longer half-life (4.5 h) than **9** (2.7 h) and **20** (3.0 h), while higher clearance was unsurprisingly observed with more lipophilic compounds (CL = 243, 238 and 128 mL/h/kg with **14**, **20** and **9**, respectively). To further improve the half-life of **14**, minimizing the amide hydrolysis was strategized by using *N,N*-disubstitution to generate steric hindrance around the amide bond. Encouragingly, the potent 3-methyl-3-hydroxyazetidinyl amide **46** had a longer half-life (6.8 h) in mice following oral administration which enabled us to combine high *in vitro* antiparasitic activity and good *in vivo* pharmacokinetic properties in a single molecule. Five additional azetidinyl amide analogs were designed and synthesized with variation of the alkyl to ethyl (**47**), *n*-propyl (**48**), isopropyl (**49**), cyclopropyl (**50**) or trifluoromethyl (**51**). In order to differentiate these six close analogs, their metabolic stabilities were assessed in an *in vitro* rat and human hepatocyte assays, which incubated 7 x 10^5 cells/well and 20 μM benzoxaborole for 44 hours. In the experiment with rat heptocytes, and as expected, an inverse relationship between metabolic stability and lipophilicity was observed. The rank order of stability was **46** (Me) > **47** (Et) > **50** (*c*-Pr) > **49** (*i*-Pr) > **48** (*n*-Pr) » **51** (CF_3). In human hepatocytes, the compounds were generally more stable than in rat hepatocytes, and the rank order was similar, e.g. **46** (Me) ≈ **47** (Et) ≈ **50** (*c*-Pr) > **49** (*i*-Pr) ≈ **48** (*n*-Pr) » **51** (CF_3). These results indicated that the 3-methyl-3-hydroxyazetidine amide **46** was most stable among the six analogs, and prompted us to further evaluate this compound in rat and

dog pharmacokinetic studies. Data from these studies is presented in Table 2. Briefly, compound **46** exhibited excellent oral exposure, low clearance and long half-life in both species.

***In Vivo* Efficacy in *P. berghei* and *P. falciparum* Mouse Models.** Compounds were selected for *in vivo* testing based on their chemical diversity, *in vitro* potency, physicochemical properties and pharmacokinetic properties. Twelve compounds (**4**, **9**, **12**, **14**, **16**, **20**, **46**, **47**, **48**, **49, 50** and **51**) were tested in a *P. berghei* mouse model.[20] In this model, compounds were dosed orally once a day for four consecutive days, beginning 1 hour after infection by intraperitoneal administration of *P. berghei*-infected erythrocytes. Compound **4** with the hydroxyethyl side chain to the amide nitrogen had a low efficacy of ED_{90} = 56.1 mg/kg. Due to the improved pharmacokinetic profiles exhibited by hydroxyethyl amides containing substituents, *in vivo* efficacy was also improved for compounds **9** (ED_{90} = 44.3 mg/kg), **14** (ED_{90} = 11.7 mg/kg) and **20** (ED_{90} = 7.3 mg/kg). Compound **16** with a *i*-Pr group attached to the side chain didn't follow this trend (ED_{90} = 26.7 mg/kg). The azetidinyl amide analogs (**46**-**51**) further improved efficacies with the ED_{90} values ranging from 1.9 to 10.3 mg/kg, and **46** had the best efficacy among these molecules, which was consistent with the result of *in vitro* metabolic stability evaluation.

Selected compounds were also tested in a *P. falciparum* mouse model, which evaluates the therapeutic efficacy of a compound against strain *Pf*3D7$^{0087/N9}$ growing in the peripheral blood of NODscidIL2Rγ^{null} mice engrafted with human erythrocytes (Table 4). Compounds were dosed orally once a day for four consecutive days. Compound **14** demonstrated excellent efficacy (ED_{90} = 2.5 mg/kg) and the corresponding exposure was AUC_{ED90} = 7.45 ug·h·mL^{-1}·day^{-1}. For the azetidinyl amide analogs (**46**, **47**, **49**, **50** and **51**), an initial test indicated that all of these five molecules were very efficacious (ED_{90} < 5 mg/kg). Based on our understanding of metabolic stability and half-life from PK experiments, the two more stable analogs (**46** and **47**) were chosen

for determination of their ED_{90} values. In these experiments, potency and exposure of **46** (ED_{90} = 0.85 mg/kg; $AUCED_{90}$ = 1.4 μg•hr/mL) and **47** (ED_{90} = 0.84 mg/kg; 1.6 μg•hr/mL) were very similar (Table 4). Furthermore, as shown in Figure 2, the result of the *P. falciparum*-infected mouse model experiment demonstrated that the *in vivo* parasite clearance profile of **46** was rapid and similar to artesunate and chloroquine, two well-known fast parasite-killing antimalarial medicines.

Since compound **46** demonstrated the best overall profile of the *in vitro* potency, *in vitro* metabolic stability, PK properties and *in vivo* efficacies amongst these amides **3-51**, it was further studied for activity against other *P. falciparum* strains, *in vitro* parasite reduction rate (PRR), and genotoxicity properties.

Since the malaria patient population includes many children and women within childbearing years, safety of antimalarial treatment has the profound priority. This research program adapted a strategy at research stage to screen representative compounds in micronucleus genetic toxicity assays. Compound **4** with a terminal hydroxyl group on the ethyl was *in vitro* micronucleus negative. It was further tested in a micronucleus *in vivo* rat model, and the result was also micronucleus negative.

Activities of Compound 46 against Multiple *P. falciparum* Parasite Strains. As summarized in Table 5, compound **46** was tested against an additional eleven *P. falciparum* strains and demonstrated excellent potency with IC_{50}'s in the range of 36-80 nM. Many of these parasite strains are chloroquine and pyrimethamine resistant, which indicated that compound **46** would not be expected to be cross resistant against these multiple drug resistant strains and it may have possible novel mechanism of action or resistance distinct from those of chloroquine and pyrimethamine.

***In Vitro P. falciparum* Parasite Killing Rate of Compound 46.** A parasite reduction ratio (PRR) *P. falciparum in vitro* assay was used to compare parasite killing rates at different concentrations. Three concentrations used were 10×, 30× and 100× IC_{50}. The assay allowed us to determine the molecule's important antiparasitic parameters such as lag phase (the time needed to show maximal killing), PRR (parasite reduction ratio or number of parasites the compound could kill in a parasite life cycle) and $PCT_{99.9\%}$ (parasite clearance time to kill 99.9% of the initial parasite population). The time-kill curves for compound **46** in Figure 3 suggested that the concentration required for **46** to achieve a maximal rate of parasite-killing was 10× to 30× IC_{50}. The data were analyzed to fit to the linear portion of the curve to determine the PRR parameters as summarized in Table 6, together with standard antimalarial agents for comparison. The result indicated that the antiparasitic rate of action of compound **46** was fast and similar to the rates for artemisinin and chloroquine.

Ames and Genetic Toxicity Test Results of Compound 46. The bacterial reverse mutation assay (Ames test) was conducted to evaluate the potential of compound **46** to induce mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 as well as the *E. coli* strain WP2 *uvr*A in the presence and absence of an exogenous metabolic activation system (S9). The results are presented in Table 7 demonstrating that molecule **46** was not mutagenic in the Ames assay under the conditions tested. The molecule was also tested in the *in vitro* micronucleus assay using human lymphocytes in the presence and absence of an Aroclor 1254 induced rat liver S9 activation system for evaluating its clastogenic potential. A dose range cytotoxicity test was performed, establishing the dose range for the definitive micronucleus assay, which was ≤ 355.15 μg/ mL of **46**. In the *in vitro* micronucleus assay, human lymphocytes were treated for 3 and 28 hours in the non-activated test system and for 3 hours in the S9 activated test system. As

shown in Table 8, in comparison with the 1% DMSO control, there were no statistically significant increases in the frequency of micronucleated cells observed in the groups treated with compound **46** under any conditions, indicating that compound **46** was not clastogenic at 3 h, with and without S9 activation, and at 28 h without S9 activation. This encouraging result led us to test compound **46** in the *in vivo* rat micronucleus model for further genotoxicity assessment by analyzing micronuclei in polychromatic erythrocyte cells (PCEs) in rat bone marrow. Three single oral doses were tested at 500, 1000 and 2000 mg/kg. The toxicokinetic plasma samples were collected and analyzed. The exposure of **46** at the highest dose of 2000 mg/kg was 742 μg×h/mL, confirming that there was adequate exposure of **46** in the in vivo micronucleus assay. There were no statistically significant increases in the incidence of micronucleated PCEs (MnPCEs) in the **46**-treated groups relative to the negative control group, and therefore, the compound did not have a micronucleus risk at all three test doses in this study (Table 9).

CONCLUSION

In summary, this lead optimization program has discovered a new potent antimalarial agent, 6-(2-((3-hydroxy-3-methylazetidin-1-yl)carbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-7-methyl-2,1-benzoxaborole (**46**). This molecule had good pharmacokinetic properties and demonstrated excellent *in vivo* efficacies against *P. falciparum* (ED_{90} = 0.85 mg/kg) and against *P. berghei* (ED_{90} = 1.92 mg/kg) in infected mice. It was highly active against various multidrug resistant *P. falciparum* strains, indicating no cross-resistance and a possible novel mechanism of action. In addition, the rapid parasite *in vitro* reduction and *in vivo* clearance profile of **46** was similar to those of artemisinin and chloroquine. Exploratory safety studies demonstrated it was Ames negative and micronucleus negative in both *in vitro* and *in vivo* models. Based on the current data, this novel benzoxaborole has been selected as a preclinical development candidate.

EXPERIMENTAL SECTION

1. **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 had ≥95% purity. Proton (^{1}H) 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 (150×4.6 mm, 5 micron, 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 1.0mL/min with a column temperature of 30 ^{o}C and UV detection at 220 nm and 254 nm. Silica gel 60 nm (200–425 mesh, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used for flash column chromatography.

2. **Care and use of experimental animals.** Pharmacokinetic studies with experimental animals were designed and performed with reference to the guidelines of the Institutional Animal Care and Use Committee (Anacor Pharmaceuticals, Inc., AN-7-2007). 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).

3. **Methods for testing *in vitro* antimalarial activity.** Experimental procedures used for determining *in vitro* antimalarial activities of the newly synthesized benzoxaboroles against a variety of *P. falciparum* parasite strains can be found in a previous report.[20]

4. **Method for pharmacokinetics measurement**. Female CD-1 mice, male SD rats, and beagle dogs received the benzoxaborole test material either by intravenous injection or by oral gavage in formulated solutions. The dose of **4**, **9**, **14**, **20** and **46** was set at 5 mg/kg for both iv and oral, and the formulation used polyethylene glycol 300, propylene glycol and water (ratio 55/25/20, pH = 6). Blood samples were collected and analyzed for drug content using HPLC coupled to tandem mass spectrometry.

5. **Method for testing *in vivo* efficacy using a *P. berghei* mouse model.** Molecules **4**, **9**, **12**, **14**, **16**, **20**, **46**, **47**, **48**, **49, 50** and **51** were tested in a *P. berghei* mouse malaria model by following the method reported previously.[20]

6. **Method for testing *in vivo* efficacy using a *P. falciparum* mouse model.** Efficacies of compounds **14**, **46**, **47**, **49**, **50** and **51** in a *P. falciparum* mouse malaria model were evaluated by using the method described previously. [21]

7. **Method of *in vitro* parasite reduction ratio (PRR) assay.** The parasite reduction ratio parameters of molecule **46** were determined by using the procedures reported previously.[22]

8. **Method for testing 46 in bacterial reverse mutation assay (Ames assay).** The study evaluated the potential of molecule **46** to induce reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and the *E. coli* strain WP2 *uvr*A in the presence and absence of an exogenous metabolic activation system (rat liver S9). The compound was

dissolved in DMSO and tested using the plate incorporation method [23] at eight dose levels (1.5, 5, 15, 50, 150, 500, 1500, 5000 μg/plate). All positive and vehicle control values were within acceptable ranges and all criteria for a valid assay were met.

9. **Methods of *in vitro* and *in vivo* micronucleus assays.** The compound was tested in the *in vitro* micronucleus assay in human lymphocytes in the presence or absence of a rat liver S9 metabolic activation system to evaluate its clastogenic/aneugenic potential. A dose range-finding assay was performed to establish the dose range for the definitive micronucleus assay. In the dose range-finding assay, human lymphocytes were treated for 3 and 28 hours in the absence of S9 and for 3 hours in the presence of S9. All cells were harvested 28 hours after initiation of test article treatment. The test article was dissolved in DMSO at concentrations up to 355.15 mg/mL (1 mM). No precipitate was observed in the treatment medium at any concentration at the beginning and the end of the treatment period. Single cultures of human lymphocytes were incubated with the compound at 0.5, 1, 2.5, 5, 10, 25, 50, 100, 200, and 355 μg/mL in all three exposure series. Selection of concentrations for the definitive micronucleus assay was based on the cytotoxicity of the test article relative to the concurrent solvent control. At least 1000 binucleated cells for each culture and 2000 for each concentration were scored. The validity of the assay was confirmed.

The *in vivo* mammalian erythrocyte micronucleus assay in rats was conducted according to established procedures.[24,25] The test article was evaluated for its clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in rat bone marrow. Carboxymethylcellulose (CMC, 1%, medium viscosity) in deionized water was used as the vehicle. Test and control article formulations were administered at a dose volume of 10 mL/kg via oral gavage. In the dose range finding assay, doses tested were 500, 1000 and 2000

mg/kg in three male rats/group. No mortality occurred at any dose. All animals appeared normal throughout the observation period. Therefore, the maximum tolerable dose for the definitive micronucleus assay was set at 2000 mg/kg, which is the maximum regulatory required dose. In the definitive assay, the study was designed to include eight groups (five rats/group): two vehicle groups for euthanasia 24 and 48 h after treatment, one 500 mg/kg/day group and one 1000 mg/kg/day for euthanasia at 24 h, two 2000 mg/kg/day groups for euthanasia at 24 and 48 h, one positive control (cyclophosphamide monohydrate, CP) group and one vinblastine positive control group for CREST analysis. Femoral bone marrow was collected at approximately 24 or 48 h after the dose. Animals were euthanized by carbon dioxide inhalation. Immediately following euthanasia, the femurs were exposed, and cut just above the knee. The bone marrow was transferred to a centrifuge tube containing approximately 2 mL fetal bovine serum and the cells were pelleted by centrifugation. The supernatant was drawn off leaving a small amount of fetal bovine serum with the pellet. Four pre-column slides for micronucleus evaluation and two post-column slides for CREST analysis were prepared from each animal. The slides were air-dried and fixed by dipping in methanol. One set of two pre-column slides was stained with acridine orange for microscopic evaluation. The other set of slides was kept as backup. Post-column slides for potential CREST staining were prepared by processing the remaining bone marrow suspensions through a cellulose column containing 0.6 g of a 50:50 mixture of cellulose type 50 and alpha-cellulose (one column per animal) and using 6 mL fetal bovine serum as the eluate. Eluted cells were pelleted by centrifugation, and the supernatant was drawn off leaving a small amount of fetal bovine serum with the pellet. The cell pellet was re-suspended for slide preparation. Two post-column slides per animal were prepared at relatively high cell density and stored at -10 to -30 ºC for CREST analysis. Each pre and post-column slide was identified by the

harvest date, study number and animal number. Slides were coded using a random number table by an individual not involved with the scoring process. Post column slides were reserved only for CREST analysis. Initially, one set of post-column slides was stained with acridine orange for micronucleus evaluation and the other set was kept as backup.

10. **Methods for the syntheses of final amide benzoxaborole compounds 3-51.** *General Method A (Substitution Method)*: This method involved a substitution reaction between the benzoxaborole intermediate **58** and an amide intermediate **120** in the final step. Representative experimental procedures are described below. 7-Methylbenzo[c][1,2]oxaborole-1,6(3H)-diol (**58**), 5-chloropyrazine-2-carboxamide (**120**, 1.1 eq), Cs_2CO_3 (2.5 eq) and DMF were added to a 3-necked flask. The reaction mixture was stirred at 40-50 ^oC until completion as monitored by HPLC. Water was added, and then HCl (0.2 N) was added dropwise to the mixture. It was stirred for 20 min while white solid was precipitated. It was filtered and the solid cake was dissolved in NaOH (1 N) and extracted by dichloromethane. The separated inorganic layer was acidified by HCl (1 N) to pH = 3, and the white solid precipitate was collected by filtration. The solid cake was stirred in water for 30 min and filtered. And then the solid was stirred in acetone and filtered. The solid was dried under high vacuum to give the desired product. If the compound didn't meet the HPLC purity requirement (≥95%), it was further purified by column chromatography so that the material reached ≥95% purity by HPLC. *General Method B (Amidation Method)*: This method included an amidation reaction from the benzoxaborole acid intermediate **123** and an amine intermediate in the final step. Representative experimental procedures are described below. To a solution of 5-chloropyrazine-2-carboxylic acid (7.9 g, 50 mmol) in MeOH (50 mL) was added $SOCl_2$ (3.0 g, 25 mmol) dropwise at 0 ^oC. The reaction mixture was stirred at 70 ^oC for 2 h, and then concentrated under the reduced pressure to give

methyl 5-chloropyrazine-2-carboxylate (4.5 g) as a white solid which was used in next step without further purification. MS (ESI+): *m/z* = 173 (M+1). A solution of this intermediate (2.9 g, 16.86 mmol), 7-methylbenzo[c][1,2]oxaborole-1,6(3H)-diol (1.94 g, 11.80 mmol) and Cs_2CO_3 (7.74 g, 23.60 mmol) in DMF (20 mL) was stirred at rt for 4 h. The mixture was poured into water (500 mL) and the solid was collected. The solid was dried under the reduced pressure to give methyl 5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxylate (2.6 g, yield 73%) as a light-brown solid. MS (ESI+): m/z = 301 (M+1). To a solution of this ester intermediate (2.6 g, 8.7 mmol) in MeOH (30 mL) was added 1N LiOH (34 mL). The reaction mixture was stirred at rt for 3 h, and then 1N HCl was added to pH = 5. The solid was collected and dried under the reduced pressure to give the acid **123** (2.3 g, yield 93%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.40 (br. s, 1H), 9.09 (s, 1H), 8.73-8.72 (m, 1H), 8.68-8.67 (m, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 5.01 (s, 2H), 2.24 (s, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; MS (ESI+): *m/z* = 287 (M+1). To a solution of **123** (100 mg, 0.35 mmol) and DIPEA (135 mg, 1.05 mmol) in DMF (3 mL) was added HATU (200 mg, 0.53 mmol). The reaction mixture was stirred at rt for 30 min, and then the corresponding amine (0.35mmol) was added. The reaction mixture was stirred at rt overnight. The crude product obtained from a normal work-up was purified by prep-HPLC to give the final product as a solid.

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(2-methoxyethyl) pyrazine-2-carboxamide (3). To a solution of 5-chloropyrazine-2-carboxylic acid (2.0g, 12.62 mmol) in DCM (42 mL) was added $SOCl_2$ (42 mL) at room temperature. The reaction was stirred at 50 ^{o}C overnight and then concentrated directly. To a solution of the residue in pyridine (42mL) was added 2-methoxyethylamine (1.69 g, 22.52 mmol, 1.78 eq) at room temperature.

The reaction was stirred at 50 °C for 3 h. The mixture was poured into water (100 mL), and then extracted with EA (3 × 50 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuum to give the residue of 5-chloro-*N*-(2-methoxyethyl)pyrazine-2-carboxamide, which was used in the next step without further purification. The final step was conducted by following the procedure described in general method A. It was obtained in 13% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.66 (s, 1H), 8.62-8.59 (m, 1H), 8.60 (s, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.25 (d, J = 8.1 Hz, 1H), 4.99 (s, 2H), 3.55-3.40 (m, 4H), 3.26 (s, 3H), 2.22 (s, 3H) ppm. HPLC purity: 98.2% at 220 nm and 96.9% at 254 nm; Mass (ESI+): *m/z* = 344 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(2-hydroxyethyl) pyrazine-2-carboxamide (4). To a solution of 5-chloropyrazine-2-carboxylic acid (500 mg, 3.16 mmol) and TEA (352 mg, 3.47 mmol, 1.1 eq) in DCM (15.8 mL) was added isobutyl chloroformate (944 mg, 6.96 mmol, 1.1 eq) at 0 °C. The reaction mixture was stirred for 20 min. Then 2-aminoethanol (231 mg, 3.79 mmol, 1.2 eq) was added at 0°C and the reaction mixture was stirred at rt for 1 h. After completion, the mixture was poured into water (100 mL) and extracted with DCM (2 × 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 give 5-chloro-N-(2-hydroxyethyl)pyrazine-2-carboxamide (480 mg, 75% yield) as a white solid. The next step was conducted by following general method A. The final product was obtained in 51% yield of the final step as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.05 (s, 1H), 8.65 (s, 1H), 8.59 (s, 1H), 8.58-8.55 (m, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 4.98 (s, 2H), 4.76 (t, J = 5.4 Hz, 1H), 3.54-3.48 (m, 2H), 3.39-3.30

(m, 2H), 2.21 (s, 3H) ppm; HPLC purity: 99.6% at 220 nm and 99.3% at 254 nm; Mass (ESI+): *m/z* = 330 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(2-hydroxypropyl) pyrazine-2-carboxamide (5). This compound was synthesized by using 2-hydroxypropylamine as a starting material and following the procedures used for the analog **3**. It was obtained in 29% yield of the final step as a solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.66 (d, J = 1.2 Hz, 1H), 8.60 (d, J = 1.2 Hz, 1H), 8.48 (t, J = 6.0 Hz, 1H), 7.29 (d, J = 8 Hz, 1H), 7.25 (d, J = 8 Hz, 1H), 4.98 (s, 2H), 4.82 (d, J = 4.8 Hz, 1H), 3.83-3.76 (m, 1H), 3.28-3.19 (m, 2H), 2.22 (s, 3H), 1.06 (d, J = 6.0 Hz, 3H) ppm. HPLC purity: 99.1% at 220 nm and 98.9% at 254 nm; Mass (ESI+): *m/z* = 344 (M+1).

***N*-(2-Hydroxy-2-methylpropyl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxa-borole-6-yloxy)pyrazine-2-carboxamide (6).** This compound was synthesized by using 2-hydroxy-2-methylpropylamine as a starting material and following the procedures used for the analog **7**. It was obtained in 68% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.68 (s, 1H), 8.64 (s, 1H), 8.25-8.40 (m, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.73 (s, 1H), 3.28 (d, J = 6.3 Hz, 2H), 2.22 (s, 3H), 1.11 (s, 6H) ppm. HPLC purity: 99.5% at 220 nm and 99.2% at 254 nm; Mass (ESI+): *m/z* = 358 (M+1).

***N*-(1-Hydroxy-2-methylpropan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborole-6-yloxy)pyrazine-2-carboxamide (7).** This compound was synthesized by using 1-hydroxy-2-methylpropan-2-yl amine as a starting material and following the procedures used for the analog **3**. It was obtained in 38% yield of the final step as a solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.65 (s, 1H), 8.57 (s, 1H), 7.94 (s, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.24

(d, *J* = 8.0 Hz, 1H), 5.13 (t, *J* = 5.0 Hz, 1H), 4.98 (s, 2H), 3.45 (d, *J* = 5.0 Hz, 2H), 2.21 (s, 3H), 1.35 (s, 6H) ppm. HPLC purity: 97.8% at 220 nm and 96.8% at 254 nm; Mass (ESI+): *m/z* = 358 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-(hydroxymethyl)cyclopropyl)pyrazine-2-carboxamide (8). This compound was synthesized by using 1-(hydroxymethyl)cyclopropylamine as a starting material and following the procedures used for the analog **3**. It was obtained in 24% yield of the final step as a solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.67 (s, 1H), 8.64 (s, 1H), 8.57 (s, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 4.71 (t, *J* = 5.7 Hz, 1H), 3.52 (d, *J* = 5.7 Hz, 2H), 2.21 (s, 3H), 0.78 (s, 4H) ppm. HPLC purity: 96.5% at 220 nm and 99.2% at 254 nm; Mass (ESI+): m/z = 356 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxypropan-2-yl)pyrazine-2-carboxamide (9). This compound was synthesized by using 2-aminopropan-1-ol as a starting material and following the procedures used for the analog **4**. It was obtained in 57% yield of the final step as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.66 (s, 1H), 8.59 (s, 1H), 8.27 (d, *J* = 8.5 Hz, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.25 (d, *J* = 8.3 Hz, 1H), 4.98 (s, 2H), 4.80 (t, *J* = 5.5 Hz, 1H), 4.10-4.00 (m, 1H), 3.50-3.40 (m, 2H), 2.22 (s, 3H), 1.15 (d, *J* = 7.0 Hz, 3H) ppm. HPLC purity: 96.5% at 220 nm and 95.1% at 254 nm; Mass (ESI+): *m/z* = 344 (M+1).

(*R*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxy propan-2-yl)pyrazine-2-carboxamide (10). This compound was synthesized by using (*R*)-2-aminopropan-1-ol as a starting material and following the procedures used for the analog **4**. It was obtained in 40% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ

9.08 (s, 1H), 8.66 (s, 1H), 8.60 (s, 1H), 8.30 (d, *J* = 8.4 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 4.83 (t, *J* = 5.5 Hz, 1H), 4.10-4.00 (m, 1H), 3.50-3.40 (m, 2H), 2.22 (s, 3H), 1.15 (d, *J* = 6.6 Hz, 3H) ppm. HPLC purity: 99.3% at 220 nm and 99.7% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): m/z = 344 (M+1).

(*S*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxy propan-2-yl)pyrazine-2-carboxamide (11). This compound was synthesized by using (*S*)-2-aminopropan-1-ol as a starting material and following the procedures used for the analog **4**. It was obtained in 10% yield of the final step as a solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.66 (s, 1H), 8.60 (s, 1H), 8.30 (d, *J* = 8.4 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 4.83 (t, *J* = 5.7 Hz, 1H), 4.10-4.00 (m, 1H), 3.50-3.40 (m, 2H), 2.22 (s, 3H), 1.15 (d, *J* = 6.6 Hz, 3H) ppm. HPLC purity: 99.3% at 220 nm and 99.6% at 254 nm; Chiral HPLC purity: 99.8% at 240 nm; Mass (ESI+): m/z = 344 (M+1).

(*R*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxy butan-2-yl)pyrazine-2-carboxamide (12). This compound was synthesized by using (*R*)-2-aminobutan-1-ol as a starting material and following the procedures used for the analog **4**. It was obtained in 59% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.66 (s, 1H), 8.61 (s, 1H), 8.23 (d, *J* = 9.0 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 4.99 (s, 2H), 4.77 (t, *J* = 5.6 Hz, 1H), 3.95-3.80 (m, 1H), 3.50-3.35 (m, 2H), 2.22 (s, 3H), 1.75-1.45 (m, 2H), 0.86 (t, *J* = 7.5 Hz, 3H) ppm. HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): *m/z* = 358 (M+1).

(*S*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-N-(1-hydroxy butan-2-yl)pyrazine-2-carboxamide (13). This compound was synthesized by using (*S*)-2-aminobutan-1-ol as a starting material and following the procedures used for the analog **4**. It was

obtained in 59% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.66 (s, 1H), 8.61 (s, 1H), 8.23 (d, J = 8.7 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 4.99 (s, 2H), 4.78 (t, J = 5.0 Hz, 1H), 3.95-3.80 (m, 1H), 3.60-3.40 (m, 2H), 2.22 (s, 3H), 1.75-1.45 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H) ppm. HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): m/z = 358 (M+1).

(*R*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxy pentan-2-yl)pyrazine-2-carboxamide (14). To a solution of (*R*)-2-aminopentanoic acid (10 g, 85.4 mmol) in THF (200 mL) was added $NaBH_4$ (10g, 85.4 mmol, 2.4 eq) at 0 ^oC under N_2. A solution of I_2 (21.7 g, 85.4 mmol, 1 eq) in THF (85 mL) was added dropwise into the mixture for 40 min until no gas was generated from the mixture. The reaction mixture was stirred overnight at 70 ^oC. After completion, methanol was added dropwise until the mixture was clear. It was added with 20% KOH (150 mL) and stirred for 4 h. The mixture was diluted with H_2O (100 mL) and extracted with DCM (3 × 150 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated to give (*R*)-2-aminopentan-1-ol (8.1 g, 92% yield). A solution of (*R*)-2-aminopentan-1-ol (6 g, 58.1 mmol) in 4 N HCl MeOH (150 mL) was stirred at rt for 40 min. The residue after rotary evaporation was mixed with 40% EA/PE (150 mL). The precipitated solid was collected by filtration to give (*R*)-2-aminopentan-1-ol HCl salt (4.6 g, 56.6% yield). To a solution of 5-chloropyrazine-2-carboxylic acid (1.13 g, 7.16 mmol) in NMP (48 mL) was added dropwise a solution of (*R*)-2-aminopentan-1-ol hydrochloride (1 g, 7.16 mmol, 1 eq) and 2-chloro-1-methylpyridinium iodide (CMPI, 4.6 g, 18 mmol, 2.5 eq) in NMP (5 mL) with stirring under N_2. The reaction was stirred at rt for 2 h. After completion, the reaction was diluted with H_2O (150 mL), adjusted to pH 3-4 with 1N HCl, and extracted with EA (2 × 150 mL). The organic phases were washed with brine (3 × 250 mL) and

dried over anhydrous Na_2SO_4. The residue after rotary evaporation was purified by column chromatography to give (*R*)-5-chloro-N-(1-hydroxypentan-2-yl)pyrazine-2-carboxamide (1.73g, 99% yield). The final step was conducted by following general method A. The product was obtained in 63% yield of the final step as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.66 (d, J = 1.0 Hz, 1H), 8.61 (d, J = 1.0 Hz, 1H), 8.24 (d, J = 9.0 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.78 (t, J = 5.5 Hz, 1H), 4.01-3.94 (m, 1H), 3.51-3.45 (m, 1H), 3.45-3.40 (m, 1H), 2.22 (s, 3H), 1.61-1.45 (m, 2H), 1.40-1.20 (m, 2H), 0.87 (t, J = 6.9 Hz, 3H) ppm. HPLC purity: 99.2% at 220 nm and 99.3% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): *m/z* = 372 (M+1).

(*S*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxypentan-2-yl)pyrazine-2-carboxamide (15). This compound was synthesized by using (*S*)-2-aminopentanoic acid as a starting material and following the procedures used for the isomer **14**. It was obtained in 31% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.66 (s, 1H), 8.60 (s, 1H), 8.22 (d, J = 9.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.77 (t, J = 5.5 Hz, 1H), 4.05-3.90 (m, 1H), 3.55-3.35 (m, 2H), 2.22 (s, 3H), 1.68-1.40 (m, 2H), 1.40-1.20 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H) ppm. HPLC purity: 99.7% at 220 nm and 99.5% at 254 nm; Mass (ESI+): *m/z* = 372 (M+1).

(*R*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (16). This compound was synthesized by using *D*-valine as a starting material and following the procedures used for the analog **14**. It was obtained in 20% yield of the final step as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.67 (s, 1H), 8.62 (s, 1H), 8.13 (d, J = 9.5 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.72 (t, J = 5.5 Hz, 1H), 3.82-3.70 (m, 1H), 3.65-3.49 (m, 2H), 2.22 (s, 3H),

1.98-1.92 (m, 1H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H) ppm. HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): m/z = 372 (M+1).

(*S*)-*N*-(1-hydroxy-3-methylbutan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (17). This compound was synthesized by using *L*-valine as a starting material and following the procedures used for the analog **14**. It was obtained in 36% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.67 (s, 1H), 8.62 (s, 1H), 8.13 (d, *J* = 9.5 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 4.72 (t, *J* = 5.5 Hz, 1H), 3.85-3.70 (m, 1H), 3.65-3.45 (m, 2H), 2.22 (s, 3H), 2.02-1.88 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H) ppm. HPLC purity: 99.8% at 220nm and 99.6% at 254nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): *m/z* = 372 (M+1).

(*R*)-*N*-(1-cyclopropyl-2-hydroxyethyl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (18). To a solution of cyclopropanecarbaldehyde (7.0 g, 99.12 mmol, 1.2 eq) in DCM (138 mL) were added $Ti(OEt)_4$ (22.6 g, 99.12mmol, 1.2 eq) and (*S*)-2-methylpropane-2-sulfinamide (10 g, 82.6 mmol) at rt. The mixture was stirred at 50 ^{o}C overnight and then was poured into saturated $NaHCO_3$ (138 mL) and diatomite (30g) with good stirring for 0.5 h. The mixture was filtered and separated. The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuum to give (*S,E*)-*N*-(cyclopropylmethylene)-2-methylpropane-2-sulfinamide (12.5g, 87% yield) as oil. To a mixture of Mg (2.86 g, 119.16 mmol, 1.65 eq) in dry THF (75 mL) were added I_2 (40 mg) and (chloromethyl)(isopropoxy)dimethylsilane (6.0 g, 36.11 mmol, 0.5 eq) under N_2. The mixture was refluxed for 1 h, and then additional (chloromethyl)(isopropoxy)dimethylsilane (12 g, 72.22

mmol, 1.0 eq) was added dropwise. The reaction mixture was refluxed for 2 h. A solution of (*S,E*)-*N*-(cyclopropylmethylene)-2-methylpropane-2-sulfinamide (12.5 g, 72.22 mmol, 1.0 eq) in dry THF (38 mL) was added to the reaction mixture at -20 ^{o}C under N_2. The mixture was stirred at rt for 1 h, poured into water (300 mL) and extracted with EA (3 × 100 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuum to give (*S*)-*N*-((*R*)-1-cyclopropyl-2-(isopropoxydimethylsilyl)ethyl)-2-methylpropane-2-sulfinamide (22.5 g, 100% yield) as oil. To a solution of this intermediate (22.3 g, 73.07 mmol) in a mixed solution [MeOH (53.5 mL) / THF (53.5 mL)] were added $KHCO_3$ (7.32 g, 73.07 mmol, 1 eq), KF (8.5 g, 146.14 mmol, 2 eq) and H_2O_2 (22.3 mL) under N_2 at 0 ^{o}C. The reaction mixture was stirred at 45 ^{o}C for 2 h, poured into water (300 mL) and extracted with EA (3 × 100mL). 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 give (*S*)-*N*-((*R*)-1-cyclopropyl-2-hydroxyethyl)-2-methylpropane-2-sulfinamide (10 g, 67% yield) as a white solid. To a solution of this intermediate (2 g, 9.75 mmol) in MeOH (50 mL) was added 1 N HCl (24 mL) at 0 ^{o}C. The reaction was stirred at room temperature for 2 h and concentrated in vacuum to give (*R*)-2-amino-2-cyclopropylethan-1-ol hydrochloride (1.3 g, 100% yield) as a white solid that was used in the next step without further purification. The subsequent reactions were conducted by following the corresponding procedures used for the analog **14**. Compound **18** was obtained in 12% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.64 (s, 1H), 8.60 (s, 1H), 8.37 (d, J = 9.0 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 4.97 (s, 2H), 4.78 (t, J = 5.5 Hz, 1H), 3.65-3.50 (m, 2H), 3.40-3.30 (m, 1H), 2.20 (s, 3H), 1.10-1.00 (m, 1H), 0.50-0.20 (m, 4H) ppm. HPLC purity: 99.6% at 220 nm and 99.7% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): m/z = 370 (M+1).

(*S*)-*N*-(1-cyclopropyl-2-hydroxyethyl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (19). This compound was prepared by following the methodology similar to that described above for **18** using (*R*)-2-methylpropane-2-sulfinamide to replace (*S*)-2-methylpropane-2-sulfinamide in the first step. Compound **19** was obtained in 6% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.66 (s, 1H), 8.61 (s, 1H), 8.37 (d, *J* = 9.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 4.79 (t, *J* = 5.5 Hz, 1H), 3.65-3.50 (m, 2H), 3.50-3.32 (m, 1H), 2.22 (s, 3H), 1.10-1.00 (m, 1H), 0.50-0.20 (m, 4H) ppm. HPLC purity: 96.6% at 220 nm and 95.9% at 254 nm; Mass (ESI+): m/z = 370 (M+1).

(*R*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(4,4,4-trifluoro-1-hydroxybutan-2-yl)pyrazine-2-carboxamide (20). To a solution of 5-chloropyrazine-2-carboxylic acid (372 mg, 2.35 mmol) and HATU (1.07 g, 2.82 mmol) in DMF (10 mL) was added DIPEA (606 mg, 4.97 mmol). The mixture was stirred at rt for 30 min, and then 2-amino-4,4,4-trifluorobutan-1-ol hydrochloride (506 mg, 2.82 mmol) was added. The resulting mixture was stirred at rt for 1 h. Water (30 mL) was added and the solution was extracted with EA (3 × 50mL). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by Combiflash (PE: EA=1:1) to give 5-chloro-N-(4,4,4-trifluoro-1-hydroxybutan-2-yl)pyrazine-2-carboxamide (435 mg, 65%) as a white solid. MS (ESI+): *m/z* = 284.0 (M+1). For the next step, the racemic compound was prepared by general method A in 57% yield of the final step. Chiral HPLC method was used for the separation of the racemic mixture and the chiral HPLC condition is shown as following: instrument SFC-80 (Thar, Waters), column CHIRALPAK AD 20*250 mm, 5 μm, column temperature 35 ^{o}C, mobile phase CO_2/Methanol = 70/30, flow rate 80g/min, back pressure 100

bar, detection wavelength 214 nm or 230 nm, cycle time 6.0 min, sample solution 300 mg in 60 mL MeOH, and injection volume 4.5 mL (loading 23 mg per injection). The separation gave the enantiomer **20** in 94.4% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.74 (d, J = 8.8 Hz, 1H), 8.67 (d, J = 1.2 Hz, 1H), 8.63 (d, J = 1.2 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 5.12 (t, J = 6.0 Hz, 1H), 4.99 (s, 2H), 4.36-4.32 (m, 1H), 3.50-3.46 (m, 1H), 3.45-3.39 (m, 1H), 2.72-2.57 (m, 2H), 2.22 (s, 3H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): m/z = 412 (M+1).

(*S*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(4,4,4-trifluoro-1-hydroxybutan-2-yl)pyrazine-2-carboxamide (21). The racemic compound was prepared by the methodology described above for **20**. Chiral HPLC separation gave the enantiomer **21** in 18.4% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.73 (d, J = 9.6 Hz, 1H), 8.67 (d, J = 1.2 Hz, 1H), 8.63 (d, J = 1.2 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 5.11 (t, J = 6.0 Hz, 1H), 4.99 (s, 2H), 4.39-4.30 (m, 1H), 3.49-3.45 (m, 1H), 3.43-3.38 (m, 1H), 2.72-2.59 (m, 2H), 2.22 (s, 3H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 98.8% at 230 nm; MS (ESI+): m/z = 412 (M+1).

(*R*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(5,5,5-trifluoro-1-hydroxypentan-2-yl)pyrazine-2-carboxamide (22). At -25 ^{o}C, 2,6-lutidine (1.41 g, 13.2 mmol) in DCM (50 mL) was mixed with Tf_2O (3.47 g, 12.3 mmol), and the mixture was stirred for 5 min. To the mixture was added 3,3,3-trifluoropropan-1-ol (1.0 g, 8.8 mmol). After 2 h, it was warmed to rt and stirred for 1h. The solvent was removed and the residue was purified by silica gel column chromatography using PE:EA = 2:1 to give 3,3,3-trifluoropropyl trifluoromethanesulfonate (460 mg, yield 21%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73-4.70 (m, 2H), 2.72-2.65 (m, 2H) ppm. A solution of *tert*-butyl 2-

(diphenylmethyleneamino)acetate (424 mg, 1.44 mmol) in THF (20 mL) was cooled to -78°C and treated dropwise with LDA (2M in THF, 1.08 mL, 2.16 mmol), and then added 3,3,3-trifluoropropyl trifluoromethanesulfonate (460 mg, 1.87 mmol) in THF (2 mL) dropwise. The reaction mixture was gradually warmed to rt and stirred for 4 h. The reaction was quenched with saturated NH_4Cl (50 mL) at 0°C and then extracted with ethyl acetate (2 × 100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using PE:EA = 40:1 to give *tert*-butyl 2-(diphenylmethyleneamino)-5,5,5-trifluoropentanoate (400 mg, yield 71%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66-7.16 (m, 10H), 3.98 (t, 1H), 2.18-2.07 (m, 4H), 1.43 (s, 9H) ppm. A solution of this intermediate (400 mg, 1.02 mmol) in 50% HCl (5 mL) was refluxed overnight. Water was removed to give 2-amino-5,5,5-trifluoropentanoic acid hydrochloride (210 mg, yield 99%) as a white solid. MS (ESI+): *m/z* =172 (M+1). To a solution of 2-amino-5,5,5-trifluoropentanoic acid hydrochloride (210 mg, 1.0 mmol) in THF (10 mL) was added LAH (76 mg, 2.0 mmol). The mixture was stirred at 70 ^{o}C overnight. Several drops of water were added and the mixture was filtered. The filtrate was concentrated under reduced pressure. 4N HCl 1,4-dioxane solution (2 mL) was added at rt and stirred for 30min. Water (10 mL) was added and then the mixture was extracted with EtOAc (2 × 10 mL). The aqueous layer was freeze-dried to give 2-amino-5,5,5-trifluoropentan-1-ol hydrochloride (150 mg, yield 77%) as a white solid. MS (ESI+): *m/z* = 158 (M+1). The subsequent reactions and chiral separation were performed by following the procedures described above for **20**. Compound **22** was obtained in yield 30% of the final step as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.67 (s, 1H), 8.62 (s, 1H), 8.49 (d, *J* = 9.2 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 4.99 (s, 2H), 4.93 (t, *J* = 6.0 Hz, 1H), 4.05-4.01 (m, 1H), 3.51-3.45 (m, 2H), 2.30-2.20 (m, 2H), 2.22 (s, 3H),

1.88-1.74 (m, 2H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm.; MS (ESI+): *m/z* = 426 (M+1).

(*S*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(5,5,5-trifluoro-1-hydroxypentan-2-yl)pyrazine-2-carboxamide (23). This (*S*)-isomer was obtained by a chiral HPLC separation from its racemic mixture as described above for **22**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.67 (s, 1H), 8.62 (s, 1H), 8.49 (d, *J* = 9.2 Hz, 2H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 1H), 4.99 (s, 2H), 4.94 (t, *J* = 6.0 Hz, 1H), 4.05-4.01 (m, 1H), 3.51-3.45 (m, 2H), 2.30-2.20 (m, 2H), 2.22 (s, 3H), 1.88-1.74 (m, 2H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 98.1% at 230 nm; MS (ESI+): *m/z* = 426 (M+1).

(*R*)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxy hexan-2-yl)pyrazine-2-carboxamide (24). This compound was synthesized by following the procedures used for the analog **14** with (*R*)-2-aminohexanoic acid as the starting material. Compound **24** was obtained in 23% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.66 (s, 1H), 8.60 (s, 1H), 8.23 (d, *J* = 9.0 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 4.99 (s, 2H), 4.77 (t, *J* = 5.5 Hz, 1H), 3.98-3.85 (m, 1H), 3.55-3.35 (m, 2H), 2.22 (s, 3H), 1.70-1.42 (m, 2H), 1.35-1.15 (m, 4H), 0.85 (m, 3H) ppm; HPLC purity: 99.2% at 220 nm and 98.9% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): *m/z* = 386 (M+1).

(*R*)-*N*-(2-hydroxy-1-phenylethyl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxa borol-6-yloxy)pyrazine-2-carboxamide (25). This compound was synthesized by using (*R*)-2-amino-2-phenylethan-1-ol as the starting material and following the procedures used for the analog **14**. Compound **25** was obtained in 33% yield of the final step as a white solid. ^{1}H NMR

(300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.92 (d, J = 8.4 Hz, 1H), 8.65 (s, 1H), 8.64 (s, 1H), 7.40-7.23 (m, 7H), 5.10-5.00 (m, 2H), 4.98 (s, 2H), 3.85-3.70 (m, 2H), 2.22 (s, 3H) ppm. HPLC purity: 99.9% at 220 nm and 99.8% at 254 nm; Chiral HPLC purity: 100% at 240 nm; Mass (ESI+): m/z = 406 (M+1).

(*R*)-*N*-(1-hydroxy-3-phenylpropan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (26). This compound was synthesized by using (*R*)-2-amino-3-phenylpropan-1-ol as a starting material and following the procedures used for the analog **14**. Compound **26** was obtained in 60% yield of the final step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.60 (s, 2H), 8.39 (d, J = 8.4 Hz, 1H), 7.33-7.22 (m, 6H), 7.21-7.13 (m, 1H), 4.98 (s, 2H), 4.93 (t, J = 5.5 Hz, 1H), 4.25-4.15 (m, 1H), 3.52-3.41 (m, 2H), 2.98-2.84 (m, 2H), 2.21 (s, 3H) ppm. HPLC purity: 98.7% at 220 nm and 100% at 254 nm; Chiral HPLC Purity: 100% at 240 nm; Mass (ESI+): m/z = 420 (M+1).

(*S*)-*N*-(1-hydroxy-3-methoxypropan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxamide (27). To a solution of benzonitrile (10 g, 97 mmol) in CH_3CH_2OH (54 g, 1164 mmol) was added acetyl chloride (61 g, 776 mmol) at 0 ^{o}C. The reaction mixture was stirred at 25 ^{o}C overnight. Aqueous $NaHCO_3$ solution was added at 0 ^{o}C until no gas was generated. The solution was extracted with ether (3 × 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give ethyl benzimidate (11g) as a crude yellow oil which was used in next step without further purification. MS (ESI+): m/z = 150 (M+1). To a solution of ethyl benzimidate (8.3 g, 55.8 mmol) in 1,2-dichloroethane (30 mL) was added (*S*)-methyl 2-amino-3-hydroxypropanoate hydrochloride (9.52 g, 61.38 mmol). The reaction mixture was stirred at 84 ^{o}C overnight. The solvent was removed under the reduced

pressure to give methyl (*S*)-2-phenyl-4,5-dihydrooxazole-4-carboxylate (10.6 g) as a crude yellow oil which was used in next step without further purification. MS (ESI+): *m/z* = 206 (M+1). To a solution of $LiAlH_4$ (2.9 g, 77.5 mmol) in THF (70 mL) was added methyl (*S*)-2-phenyl-4,5-dihydrooxazole-4-carboxylate (10.6 g, 51.7 mmol) in THF (70mL) at 0 ^oC. The reaction mixture was stirred at 70 ^oC for 2 h, and then water was added dropwise until no gas was generated. It was filtered and the filtering cake was washed with EA. The combined organic phase was concentrated in vacuum and the residue was purified by Combiflash (EA) to give (*R*)-(2-phenyl-4,5-dihydrooxazol-4-yl)methanol (2.0 g, 16%, over 3 steps) as a white solid. MS (ESI+): *m/z* = 178 (M+1). To a solution of this intermediate (2.0 g, 11.2 mmol) in THF (60 mL) was added NaH (542 mg, 22.4 mmol) at 0 ^oC. After being stirred at 0 ^oC for 15 min, CH_3I (3.98 g, 28.0 mmol) was added. The reaction mixture was stirred at rt overnight. The solvent was removed and the residue was purified by silica gel column chromatography using PE: EA = 5:1 to give (*R*)-4-(methoxymethyl)-2-phenyl-4,5-dihydrooxazole (1.9 g, yield 88%) as a white solid. MS (ESI+): *m/z* = 192 (M+1). The solution of this intermediate (1.9 g, 11.8 mmol) in 4N HCl (60 mL) was refluxed overnight. After cooled to rt, it was filtered and the filtrate washed by ether (3 × 50 mL). The water layer was lyophilizated to give (*S*)-2-amino-3-methoxypropan-1-ol hydrochloride (1.5 g, yield 83%) as a white solid. MS (ESI+): *m/z* = 106 (M+1). The subsequent reactions were performed by following the procedures described above for **20**. Compound **27** was acquired in 52.5% yield of the final step as a white solid. ^1H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.67 (d, *J* = 1.2 Hz, 1H), 8.62 (d, *J* = 1.2 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 9.0 Hz, 1H), 7.26 (d, *J* = 9.0 Hz, 1H), 4.99 (s, 2H), 4.91 (t, *J* = 5.2 Hz, 1H), 4.17-4.09 (m, 1H), 3.55-3.44 (m, 4H), 3.27 (s, 3H), 2.22 (s, 3H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 200 nm; MS (ESI+): m/z = 374 (M+1).

(*S*)-*N*-(1-hydroxy-3-(methylthio)propan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxamide (28). To a solution of (*S*)-2-amino-3-mercaptopropanoic acid hydrochloride hydrate (3.0 g, 17.0 mmol) in CH_3OH (50 mL) was added $SOCl_2$ (4.1 g, 34.0 mmol) at 0 ^oC. The reaction mixture was stirred at 60 ^oC overnight. It was concentrated under the reduced pressure to give (*S*)-methyl 2-amino-3-mercaptopropanoate hydrochloride (2.3 g) as a white solid, which was used in next step without further purification. MS (ESI+): *m/z* = 136 (M+1). To a solution of this intermediate (2.0 g, 11.4 mmol) in DCM (50 mL) was added $(Boc)_2O$ (3.7 g, 17.1 mmol). The reaction mixture was stirred at rt for 2 h. After being cooled to rt, EA (150 mL) and ammonia water (50 mL) were added. The organic layer after separation was dried over Na_2SO_4, filtered and concentrated under a reduced pressure to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-mercaptopropanoate (4 g) as a white solid. MS (ESI+): *m/z* = 136 (M-99). To a solution of this intermediate (4.0 g, 17.0 mmol) in DCM (50 mL) was added MeI (4.8 g, 34.0 mmol) and DIPEA (4.4 g, 34.0 mmol). The reaction mixture was stirred at 25 ^oC overnight. The solvent was removed and the residue was purified by silica gel column chromatography using PE: EA = 2:1 to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(methylthio)propanoate (2.34g, yield 54%) as a colorless oil. MS (ESI+): *m/z* = 150 (M-99). To a solution of this intermediate in 1,4-dioxane (30 mL) was added 4N HCl 1,4-dioxane solution (30 mL). The reaction mixture was stirred at 25 ^oC overnight and concentrated under the reduced pressure to give crude (*S*)-methyl 2-amino-3-(methylthio)propanoate hydrochloride (1.4 g) as a white solid that was used in next step without further purification. MS (ESI+): *m/z* = 150 (M+1). To a solution of $LiAlH_4$ (0.53 g, 14.0 mmol) in THF (25 mL) was added a solution of this intermediate (1.4 g, 9.3 mmol) in THF (5 mL) at 0 ^oC. The reaction mixture was stirred at 70 ^oC for 2 h, and then water was added dropwise until no

gas was generated. It was filtered off and the filtered cake was washed with EA. The combined organic phase was concentrated in vacuum. Then 4N HCl/1,4-dioxane solution (20 mL) was added and the resulting mixture was stirred at rt for 30 min. After removing the solvent, water (30 mL) was added and it was extracted with EA (3 × 30 mL) to remove organically soluble impurities. The water layer was lyophilized to give (*S*)-2-amino-3-(methylthio)propan-1-ol hydrochloride (880 mg, yield 58%) as a colorless oil. MS (ESI+): *m/z* = 122 (M+1). The subsequent reactions were performed by following the procedures described above for **20**. Compound **33** was acquired in 47% yield of the final step as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.68 (s, 1H), 8.63 (s, 1H), 8.41 (d, J = 9.2 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 4.99 (s, 2H), 4.95 (t, J = 5.2 Hz, 1H), 4.16-4.08 (m, 1H), 3.62-3.47 (m, 2H), 2.79-2.65 (m, 2H), 2.22 (s, 3H), 2.08 (s, 3H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): *m/z* = 390 (M+1).

(*S*)-*N*-(1-hydroxy-3-(methylsulfonyl)propan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxamide (29). To a solution of (*S*)-5-chloro-N-(1-hydroxy-3-(methylthio)propan-2-yl)pyrazine-2-carboxamide (100 mg, 0.383 mmol), obtained during the synthesis of **28**, in DCM (10 mL) was added *m*-CPBA (132 mg, 0.766 mmol) at 0 ^{o}C. The mixture was stirred at rt for 90 min. Water (20 mL) was added and the mixture was extracted with DCM (2 × 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by Combiflash (PE:EA = 1:8) to give ((*S*)-5-chloro-N-(1-hydroxy-3-(methylsulfonyl)propan-2-yl)pyrazine-2-carboxamide (89 mg, yield 79%) as a yellow solid. MS (ESI+): *m/z* = 294 (M+1). The last step was conducted by following general method A. Compound **29** was obtained in 45% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ

9.10 (s, 1H), 8.78 (d, *J* = 9.2 Hz, 1H), 8.68 (d, *J* = 1.4 Hz, 1H), 8.64 (d, *J* = 1.4 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 5.15 (t, *J* = 5.6 Hz, 1H), 4.99 (s, 2H), 4.60-4.45 (m, 1H), 3.61-3.31 (m, 4H), 2.97 (s, 3H), 2.22 (s, 3H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral purity: 100% at 230 nm; MS (ESI+): *m/z* = 422 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(3-hydroxybutan-2-yl)pyrazine-2-carboxamide (30). The solution of 3-nitrobutan-2-ol (1.0 g, 8.4 mmol) in MeOH (30 mL) was hydrogenated using 10% Pd/C (100 mg) as catalyst under H_2 overnight. The catalyst was removed by filtration through Celite and the solvent was evaporated under reduced pressure to give 3-aminobutan-2-ol (740 mg, yield 99%) as colorless oil. The next step was performed by following general method B. Compound **30** was acquired in 41% yield as a white solid. ^{1}H NMR (400MHz, DMSO-d_6): δ 9.11 (s, 1H), 8.67-8.66 (m, 1H), 8.62-8.60 (m, 1H), 8.32 (d, *J* = 9.2 Hz, 1H), 8.10 (d, *J* = 9.2 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 3.90-3.85 (m, 1H), 3.73-3.64 (m, 1H), 2.22 (s, 3H), 1.14 (dd, J_1 = 12.0 Hz, J_2 = 6.8 Hz, 3H), 1.05 (dd, J_1 = 11.6 Hz, J_2 = 6.4 Hz, 3H) ppm; HPLC purity: 99.0% at 220 nm and 100% at 254 nm; MS (ESI+): *m/z* = 358 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-((*1S,2S*)-2-hydroxycyclopentyl)pyrazine-2-carboxamide (31). This compound was prepared by general method B from (*1S,2S*)-2-aminocyclopentanol and the acid **123**. It was obtained in 15% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.65 (d, *J* = 0.8 Hz, 1H), 8.60 (d, *J* = 0.8 Hz, 1H), 8.48 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 4.79 (d, *J* = 4.4 Hz, 1H), 4.04-4.00 (m, 2H), 2.21 (s, 3H), 1.99-1.95 (m, 1H), 1.86-1.83 (m, 1H), 1.67-1.62 (m, 2H), 1.54-1.46 (m, 2H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): *m/z* = 370 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-((*1R*,*2R*)-2-hydroxycyclopentyl)pyrazine-2-carboxamide (32). This compound was prepared by general method B from (*1R,2R*)-2-aminocyclopentanol and the acid **123**. It was obtained in 31% yield as a white solid. ^{1}H NMR (400MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.65 (d, J = 1.2 Hz, 1H), 8.61 (d, J = 1.2 Hz, 1H), 8.48 (d, J = 7.2 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.03-4.00 (m, 2H), 2.21 (s, 3H), 1.99-1.95 (m, 1H), 1.86-1.83 (m, 1H), 1.67-1.61 (m, 2H), 1.54-1.45 (m, 2H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): *m/z* = 370 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(*cis*-2-hydroxycyclopentyl)pyrazine-2-carboxamide (33). This compound was prepared by general method B from *cis*-2-aminocyclopentan-1-ol and the acid **123**. It was obtained in 18% yield as a white solid. The ratio of these two *cis*-isomers in the racemic product was 50.2 : 49.8 by chiral HPLC analysis. ^{1}H NMR (400MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.67 (d, J = 0.8 Hz, 1H), 8.62 (d, J = 0.8 Hz, 1H), 8.18 (d, J = 7.6 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 5.12 (d, J = 4.4 Hz, 1H), 4.99 (s, 2H), 4.10-4.00 (m, 2H), 2.21 (s, 3H), 2.00-1.85 (m, 1H), 1.85-1.70 (m, 2H), 1.70-1.50 (m, 3H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; MS (ESI+): *m/z* = 370 (M+1).

(*S*)-*N*-(3-Hydroxy-3-methylbutan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxamide (34). To a solution of (*S*)-2-aminopropanoic acid (1.0 g, 11.23 mmol) in MeOH (30 mL) at 0 ^{o}C was added dropwise $SOCl_2$ (2.67 g, 22.47 mmol). The reaction mixture was refluxed for 2 h, and then the solvent was removed to give crude (*S*)-methyl 2-aminopropanoate hydrochloride (1.6 g) as a white solid. To a solution of this intermediate (1.6 g, 11.51 mmol) in THF (30 mL) at 0 ^{o}C was added MeMgBr (3N, 15 mL,

46.04 mmol) dropwise. The reaction mixture was stirred at rt overnight. The mixture was quenched with saturated NH_4Cl and extracted with EA (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with MeOH: DCM: Et_3N (10:80:3) to give crude (*S*)-3-amino-2-methylbutan-2-ol (100 mg) as a colorless oil. MS (ESI+): m/z = 104 (M+1). The subsequent reaction of this intermediate with the acid **123** was conducted by general method B. Compound **34** was obtained in 11% yield as a white solid. ^{1}H NMR (400MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.67 (d, J = 1.2 Hz, 1H), 8.62 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.72 (s, 1H), 3.92-3.87 (m, 1H), 2.22 (s, 3H), 1.15 (s, 3H), 1.12 (d, J = 6.4 Hz, 3H), 1.09 (s, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): *m/z* = 372 (M+1).

(*R*)-*N*-(3-Hydroxy-3-methylbutan-2-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (35). This compound was prepared by starting from (*R*)-2-aminopropanoic acid and following the procedures described above for **34**. Compound **35** was acquired in 10% yield as a white solid. ^{1}H NMR (400MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.67 (d, J = 1.2 Hz, 1H), 8.62 (s, 1H), 8.12 (d, J = 9.2 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.72 (s, 1H), 3.92-3.87 (m, 1H), 2.22 (s, 3H), 1.15 (s, 3H), 1.12 (d, J = 6.4 Hz, 3H), 1.09 (s, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 220 nm; MS (ESI+): *m/z* = 372 (M+1).

(*S*)-*N*-(2-Hydroxy-2-methylpentan-3-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (36). This compound was prepared by starting from (*S*)-2-aminobutanoic acid and following the procedures described above for **34**. Compound **36**

was obtained in 10% yield as a white solid. ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.68 (d, J = 1.2 Hz, 1H), 8.62 (d, J = 1.2 Hz, 1H), 7.98 (d, J = 10.4 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 4.99 (s, 2H), 4.59 (s, 1H), 3.75 (td, J_t = 10.4 Hz, J_d = 2.2 Hz, 1H), 2.23 (s, 3H), 1.79-1.76 (m, 1H), 1.47-1.45 (m, 1H), 1.15 (s, 3H), 1.06 (s, 3H), 0.81 (t, J = 7.4 Hz, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): m/z = 386 (M+1).

(*R*)-*N*-(2-Hydroxy-2-methylpentan-3-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (37). This compound was prepared by starting from (*R*)-2-aminobutanoic acid and following the procedures described above for **34**. Compound **37** was acquired in 18% yield as a white solid. ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.68 (d, J = 1.2 Hz, 1H), 8.62 (d, J = 1.2 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 4.98 (s, 2H), 4.59 (s, 1H), 3.74 (td, J_t = 10.8 Hz, J_d = 2.8 Hz, 1H), 2.22 (s, 3H), 1.80-1.74 (m, 1H), 1.48-1.42 (m, 1H), 1.14 (s, 3H), 1.05 (s, 3H), 0.80 (t, J = 7.6 Hz, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): m/z = 386 (M+1).

(*S*)-*N*-(2-Hydroxy-2-methylhexan-3-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazine-2-carboxamide (38). This compound was prepared by starting from (*S*)-2-aminopentanoic acid and following the procedures described above for **34**. Compound **38** was obtained in 10% yield as a white solid. ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.07 (br. s, 1H), 8.66 (s, 1H), 8.61 (s, 1H), 7.98 (d, J = 10.0 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 4.97 (s, 2H), 4.60 (s, 1H), 3.83 (t, J = 9.4 Hz, 1H), 2.22 (s, 3H), 1.69-1.61 (m, 1H), 1.50-1.45 (m, 1H), 1.34-1.16 (m, 2H), 1.13 (s, 3H), 1.04 (s, 3H), 0.84 (t, J = 7.4 Hz, 3H) ppm. HPLC

purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): *m/z* = 400 (M+1).

(*R*)-*N*-(2-Hydroxy-2-methylhexan-3-yl)-5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carboxamide (39). This compound was prepared by starting from (*R*)-2-aminopentanoic acid and following the procedures described above for **34**. Compound **39** was obtained in 10% yield as a white solid. ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.66 (d, J = 1.2 Hz, 1H), 8.61 (d, J = 1.2 Hz, 1H), 7.97 (d, J = 10.4 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 4.98 (s, 2H), 4.58 (s, 1H), 3.84 (td, J_t = 10.8 Hz, J_d = 2.4 Hz, 1H), 2.22 (s, 3H), 1.70-1.62 (m, 1H), 1.52-1.42 (m, 1H), 1.34-1.16 (m, 2H), 1.14 (s, 3H), 1.05 (s, 3H), 0.85 (t, J = 7.6 Hz, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): *m/z* = 400 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(2-hydroxyethyl)-N-methylpyrazine-2-carboxamide (40). This compound was synthesized by using 2-(methylamino)ethanol as a starting material and following the procedures used for the analog **4**. Compound **40** was obtained in 26% yield of the last step as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.60-8.45 (m, 1H), 8.40-8.25 (m, 1H), 7.35-7.20 (m, 2H), 4.98 (s, 2H), 4.82-4.62 (m, 1H), 3.70-3.30 (m, 4H), 3.12-2.95 (m, 3H), 2.24 (s, 3H) ppm. HPLC purity: 100% at 220 nm and 98.0% at 254 nm; Mass (ESI+): *m/z* = 344 (M+1).

(*R*)-5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*-(1-hydroxy pentan-2-yl)-*N*-methylpyrazine-2-carboxamide (41). To a solution of (*R*)-methyl 2-aminopentanoate hydrochloride (1.6 g) and Et_3N (3.7 mL, 25.65 mmol) in DCM (20 mL) was added $(Boc)_2O$ (3.7 g, 17.09 mmol). The reaction mixture was stirred at rt overnight. Solvent was removed and the residue was purified by silica gel column chromatography using PE:EA (8:1) as

eluent to give (*R*)-methyl 2-(*tert*-butoxycarbonylamino)pentanoate (1.8 g, yield 91%) as a colorless oil. MS (ESI+): *m/z* = 254 (M+23). To a solution of this intermediate (500 mg, 2.16 mmol) in THF (30 mL) was added $LiAlH_4$ (247 mg, 6.49 mmol). The reaction mixture was refluxed overnight. Water (0.3 mL) was added and the solid was removed by filtration. The filtrate was concentrated and the residue was purified by silica gel column chromatography using DCM: MeOH: Et_3N (80:10:3) as eluent to give (*R*)-2-(methylamino)pentan-1-ol (100 mg) as a colorless oil. MS (ESI+): *m/z* = 118 (M+1). The subsequent reaction of this intermediate with the acid **123** was conducted by general method B. Compound **41** was obtained in 35% yield as a white solid. ^{1}H NMR (400MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.55 (d, *J* = 1.0 Hz, 0.34H), 8.53 (d, *J* = 1.0 Hz , 0.66H), 8.30 (d, *J* = 1.0 Hz, 0.34H), 8.23 (d, *J* = 1.0 Hz , 0.66H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 4.98 (s, 2H), 4.77-4.74 (m, 1H), 4.60-4.50 (m, 0.35H), 3.75-3.65 (m, 0.65H), 3.55-3.31 (m, 2H), 2.83 (s, 3H), 2.23 (s, 3H), 1.48-1.23 (m, 4H), 0.91 (t, *J* = 7.2 Hz, 1H), 0.82 (t, *J* = 7.2 Hz, 2H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral purity 100% at 230 nm; MS (ESI+): *m/z* = 386 (M+1).

5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)-*N*,*N*-bis(2-hydroxy ethyl)pyrazine-2-carboxamide (42). This compound was synthesized by using diethanolamine as a starting material and following the procedures described for **4**. Compound **42** was obtained in 8% yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.49 (s, 1H), 8.30 (s, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 4.97 (s, 2H), 4.76 (t, *J* = 5.0 Hz, 1H), 4.66 (s, 1H), 3.61-3.59 (m, 2H), 3.54-3.51 (m, 2H), 3.49 (s, 4H), 2.22 (s, 3H) ppm. HPLC purity: 93.3% at 220 nm and 96.7% at 254 nm; Mass (ESI+): *m/z* = 374 (M+1).

(*S*)-(5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazin-2-yl)(2-(hydroxymethyl)piperazin-1-yl)methanone Hydrochloric Acid Salt (43). General method B

was used for the reaction of (*S*)-*tert*-butyl 3-(hydroxymethyl)piperazine-1-carboxylate with the acid **123** providing (*S*)-*tert*-butyl 4-(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carbonyl)-3-(hydroxymethyl)piperazine-1-carboxylate as a white solid in 38% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.53 (s, 1H), 8.34-8.31 (m, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 8.0 Hz, 1H), 4.97 (s, 2H), 4.90-4.80 (m, 1H), 4.22-4.19 (m, 1H), 4.04-3.71 (m, 3H), 3.49 (s, 1H), 3.21-2.92 (m, 4H), 2.23 (s, 3H), 1.39 (s, 9H) ppm; MS (ESI+): *m/z* = 429 (M-55). This Boc-protected intermediate was deprotected with 4N HCl 1,4-dioxane solution in DCM generating the residue, which was purified by prep-HPLC to give compound **43** in 13% yield as a white solid. HPLC purity: 98.4% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 98.7% at 230 nm; MS (ESI+): *m/z* = 385 (M+1).

(*R*)-(5-(1-Hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazin-2-yl)(2-(hydroxymethyl)piperazin-1-yl)methanone Hydrochloric Acid Salt (44). General method B was used for the reaction of (*R*)-*tert*-butyl 3-(hydroxymethyl)piperazine-1-carboxylate with the acid **123** providing (*R*)-*tert*-butyl 4-(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazine-2-carbonyl)-3-(hydroxymethyl)piperazine-1-carboxylate in 38% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.53 (s, 1H), 8.35-8.29 (m, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 4.97 (s, 2H), 4.90-4.79 (m, 1H), 4.23-4.20 (m, 1H), 4.02-3.68 (m, 3H), 3.48 (s, 2H), 3.05-2.91 (m, 3H), 2.23 (s, 3H), 1.39 (s, 9H) ppm; MS (ESI+): *m/z* = 429 (M-55). This Boc-protected intermediate was deprotected with 4N HCl 1,4-dioxane solution in DCM generating the residue, which was purified by prep-HPLC to give compound **44** in 12% yield as a white solid. HPLC purity: 100% at 214 nm and 100% at 254 nm; Chiral HPLC purity: 100% at 230 nm; MS (ESI+): *m/z* = 385 (M+1).

(3-Hydroxy-3-methylpyrrolidin-1-yl)(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazin-2-yl)methanone (45). This compound was prepared from 3-methylpyrrolidin-3-ol hydrochloride and the acid **123** by general method B. Compound **45** was obtained in 12% yield as a white solid. The ratio of the two stereo-isomers in this racemic product was about 60:40 by ^{1}HNMR data. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.57-8.56 (m, 1H), 8.50-8.48 (m, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 4.99 (s, 2H), 4.85 (s, 0.4H), 4.79 (s, 0.6H), 3.80-3.49 (m, 4H), 2.23 (s, 3H), 1.85-1.80 (m, 2H), 1.33 (s, 1.2H), 1.27 (s, 1.8H) ppm; HPLC purity: 100% at 220 nm and 100% at 254 nm; MS (ESI+): m/z = 370 (M+1).

(3-Hydroxy-3-methylazetidin-1-yl)(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazin-2-yl)methanone (46). To a solution of 1-benzhydrylazetidin-3-one (7.11 g, 30 mmol) in dry THF (150 mL) was added methylmagnesium bromide (3N, 30 mL, 90 mmol) dropwise at -78 ^{o}C. After being stirred at -78 ^{o}C for 2 h, the reaction mixture was gradually warmed to rt and stirred overnight. Saturated NH_4Cl (100 mL) was added and extracted with EA (3 × 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under the reduced pressure. The residue was purified by silica gel column chromatography eluted with PE:EA (4:1) to give 1-benzhydryl-3-methylazetidin-3-ol (7.0 g, yield 92%) as a yellow solid. MS (ESI+): m/z = 254 (M+1). The solution of this intermediate (7.0 g, 27.7 mmol) and HCl (1N, 28mL) in MeOH (100 mL) was hydrogenated using 10% Pd/C (800 mg) as catalyst under atmospheric pressure of H_2 overnight. The catalyst was removed and the solvent was evaporated to give 3-methylazetidin-3-ol hydrochloride (3.0 g, yield 88%) as a light yellow solid. The subsequent reaction of this intermediate with the acid **123** was performed by using general method B to provide compound

46 in 47% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.61 (d, J=1.2 Hz, 1H), 8.56 (d, J = 1.2 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 5.70 (s, 1H), 4.99 (s, 2H), 4.41 (d, J = 9.6 Hz, 1H), 4.35 (d, J = 9.6 Hz, 1H), 3.95 (d, J = 9.6 Hz, 1H), 3.90 (d, J = 9.6 Hz, 1H), 2.21 (s, 3H), 1.40 (s, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; MS (ESI+): m/z = 356 (M+1).

(3-Ethyl-3-hydroxyazetidin-1-yl)(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazin-2-yl)methanone (47). This compound was prepared by using ethylmagnesium bromide as a starting material and following the procedures described above for **46**. Compound **47** was obtained in 37% yield of the last step as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.60 (d, J = 1.2 Hz, 1H), 8.55 (d, J = 1.2 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 5.60 (s, 1H), 4.98 (s, 2H), 4.40 (d, J = 10.0 Hz, 1H), 4.29 (d, J = 10.0 Hz, 1H), 3.95 (d, J = 10.0 Hz, 1H), 3.83 (d, J=10.0 Hz, 1H), 2.20 (s, 3H), 1.65 (q, J = 7.2 Hz, 2H), 0.87 (t, J = 7.2 Hz, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; MS (ESI+): m/z = 370 (M+1).

(3-Hydroxy-3-propylazetidin-1-yl)(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazin-2-yl)methanone (48). This compound was prepared by using propylmagnesium chloride as a starting material and following the procedures described above for **46**. Compound **48** was obtained in 36% yield of the final step as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.61 (d, J = 0.8 Hz, 1H), 8.56 (d, J = 0.8 Hz, 1H), 7.29 (d, J = 6.4 Hz, 1H), 7.25 (d, J = 6.4 Hz, 1H), 5.60 (s, 1H), 4.99 (s, 2H), 4.41 (d, J = 8.0 Hz, 1H), 4.31 (d, J = 8.0 Hz, 1H), 3.96 (d, J = 8.4 Hz, 1H), 3.85 (d, J = 8.4 Hz, 1H), 2.22 (s, 3H), 1.63 (t, J = 6.4 Hz, 2H), 1.39-1.35 (m, 2H), 0.90 (t, J = 6.0 Hz, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; MS (ESI+): m/z = 384 (M+1).

(3-Hydroxy-3-isopropylazetidin-1-yl)(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2] oxaborol-6-yloxy)pyrazin-2-yl)methanone (49). This compound was prepared by using isopropylmagnesium chloride as a starting material and following the procedures described above for **46**. Compound **49** was obtained in 36% yield of the last step as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.62 (d, J = 1.2 Hz, 1H), 8.58 (d, J = 1.2 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 5.56 (s, 1H), 4.99 (s, 2H), 4.45 (d, J = 10.4 Hz, 1H), 4.27 (d, J = 10.4 Hz, 1H), 4.01 (d, J = 10.4 Hz, 1H), 3.80 (d, J = 10.4 Hz, 1H), 2.21 (s, 3H), 1.85-1.82 (m, 1H), 0.87-0.85 (m, 6H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; MS (ESI+): m/z = 384 (M+1).

(3-Cyclopropyl-3-hydroxyazetidin-1-yl)(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazin-2-yl)methanone (50). This compound was prepared by using cyclopropylmagnesium bromide as a starting material and following the procedures described above for **46**. Compound **50** was obtained in 32% yield of the last step as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.61 (d, J = 1.2 Hz, 1H), 8.56 (d, J = 1.2 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 5.66 (s, 1H), 4.99 (s, 2H), 4.35 (d, J = 10.4 Hz, 1H), 4.29 (d, J = 10.4 Hz, 1H), 3.89 (d, J = 10.4 Hz, 1H), 3.82 (d, J = 10.4 Hz, 1H), 2.22 (s, 3H), 1.22-1.18 (m, 1H), 0.43-0.40 (m, 2H), 0.34-0.31 (m, 2H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; MS (ESI+): m/z = 382 (M+1).

(3-Hydroxy-3-(trifluoromethyl)azetidin-1-yl)(5-(1-hydroxy-7-methyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yloxy)pyrazin-2-yl)methanone (51). To a solution of 1-benzhydrylazetidin-3-one (2.0 g, 8.45 mmol) in THF (25 mL) was added trimethyl(trifluoromethyl)silane (1.80 g, 12.65 mmol) and cesium fluoride (1.95 g, 12.85 mmol). The reaction mixture was stirred at rt for 1 h and quenched with saturated NH_4Cl solution. The

mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuum. The crude product was purified by column chromatography on silica gel using PE:EA (4:1) as eluent to give 1-benzhydryl-3-(trifluoromethyl)azetidin-3-ol (1.55 g, yield 60%) as a yellow solid. MS (ESI+): *m/z* = 308 (M+1). The subsequent reactions were conducted by following the procedures described above for **46**. Compound **51** was obtained in 25% yield of the last step as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.65 (d, J = 1.4 Hz, 1H), 8.57 (d, J = 1.4 Hz, 1H), 7.48 (s, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 4.98 (s, 2H), 4.80 (d, J = 11.1 Hz, 1H), 4.55 (d, J = 11.1 Hz, 1H), 4.31 (d, J = 11.1 Hz, 1H), 4.07 (d, J = 11.1 Hz, 1H), 2.21 (s, 3H) ppm; HPLC purity: 100% at 214 nm and 100% at 254 nm; MS (ESI+): *m/z* = 410 (M+1).

7-Methylbenzo[c][1,2]oxaborole-1,6(3H)-diol (58). Phosphorous oxychloride (11 mL, 118.3 mmol, 2.33 eq) was added dropwise to DMF (150 mL) stirring at 0 °C in a round-bottom flask under N_2, and the mixture was stirred at rt for 0.5 h. It was transferred via cannula to a solution of 2-methylbenzene-1,3-diol (7.0 g, 50.7 mmol) in DMF (100 mL) stirring at 0 °C under N_2. The mixture was stirred for 1.5 h and was slowly warmed to rt. It was poured into ice-water (2 L) and neutralized with 2N NaOH to pH = 6. The mixture was extracted with EA (3 × 600 mL) and the organic layer was dried over Na_2SO_4, filtered and concentrated in vacuum. The resulting residue was purified by column chromatography to give 2,4-dihydroxy-3-methylbenzaldehyde (5.0 g, 63% yield) as a yellow solid. To the mixture of this intermediate (5.0 g, 30 mmol), $NaHCO_3$ (3.3 g, 39 mmol, 1.3 eq) and KI (996 mg, 6 mmol, 0.2 eq) in MeCN (80 mL) was added BnBr (4.3 mL, 36.14 mmol, 1.2 eq) at 60 ^{o}C. The mixture was stirred at 80 ^{o}C overnight, cooled to rt and evaporated. The residue was quenched with 2N HCl (200 mL) and extracted with EA (3 × 60 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and

concentrated in vacuum. The residue was purified by column chromatography to give 4-(benzyloxy)-2-hydroxy-3-methylbenzaldehyde (5.0 g, 65% yield) as a light yellow solid. To a solution of this intermediate (11.5 g, 44.87 mmol) and pyridine (18 mL, 224.35 mmol, 5 eq) in DCM (224 mL) was added dropwise a solution of Tf_2O (15 mL, 89.74 mmol, 2 eq) in DCM (30 mL) at 0 ^oC. The reaction mixture was stirred at rt for 1 h, added with 0.5N HCl (100 mL) and extracted with DCM (3 × 30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography to give 3-(benzyloxy)-6-formyl-2-methylphenyl trifluoromethanesulfonate (11 g, 65% yield) as oil. To the mixture of this intermediate (10 g, 25.75 mmol), Pin_2B_2 (7.8 g, 30.90 mmol, 1.2 eq) and KOAc (7.57 g, 77.25 mmol, 3 eq) in 1,4-dioxane (130 mL) was added $Pd(dppf)Cl_2$ (2 g, 2.575 mmol, 0.1 eq). The reaction mixture was stirred at 95 ^oC under N_2 overnight, poured into water (1.2 L) and extracted with EA (3 × 400 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 give 4-(benzyloxy)-3-methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (5.1 g, 54% yield) as oil. To a solution of this intermediate (1.7 g, 4.64 mmol) in MeOH (23 mL) was added $NaBH_4$ (176 mg, 4.64 mmol, 1 eq). The reaction was stirred at rt for 1 h and acidified with 2N HCl (6 mL) to pH = 2. It was stirred at rt for additional 1 h, poured into water (100 mL) and extracted with EA (3 × 30 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 give 6-(benzyloxy)-7-methylbenzo[c][1,2]oxaborol-1(3H)-ol (942 mg, 80% yield) as a white solid. The solution of this intermediate (1.0 g, 3.73 mmol) in MeOH (19 mL) was hydrogenated overnight using 10% Pd/C (0.2 g) as a catalyst. The catalyst was removed by filtration through Celite and

the solvent was evaporated under reduced pressure. The residue was purified by column chromatography to give the desired compound **58** (0.4 g, 61% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.03 (s, 1H), 8.76 (s, 1H), 6.98 (d, J = 8.1 Hz, 1H), 6.88 (d, J = 8.1 Hz, 1H), 4.83 (s, 2H), 2.25 (s, 3H) ppm; HPLC purity: 97.3% at 220 nm. Compound **58** was used as a common intermediate for the syntheses of **3**-**51**.

ASSOCIATED CONTENT

Supporting Information

Figures illustrating mice survival and efficacy ED_{90} data from a *P. berghei* mouse malaria model and molecular formula strings with the associated biochemical and biological data as a CSV file are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: yongkangzhang@yahoo.com.

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ABBREVIATIONS

NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography; ESI , electrospray ionization; rt, room temperature; 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-dimethylaminopropyl)carbodiimide; NMM, *N*-methylmorpholine.

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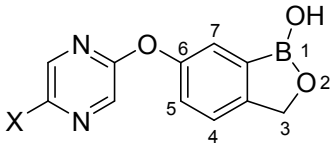
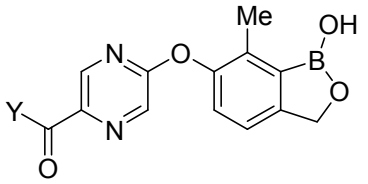


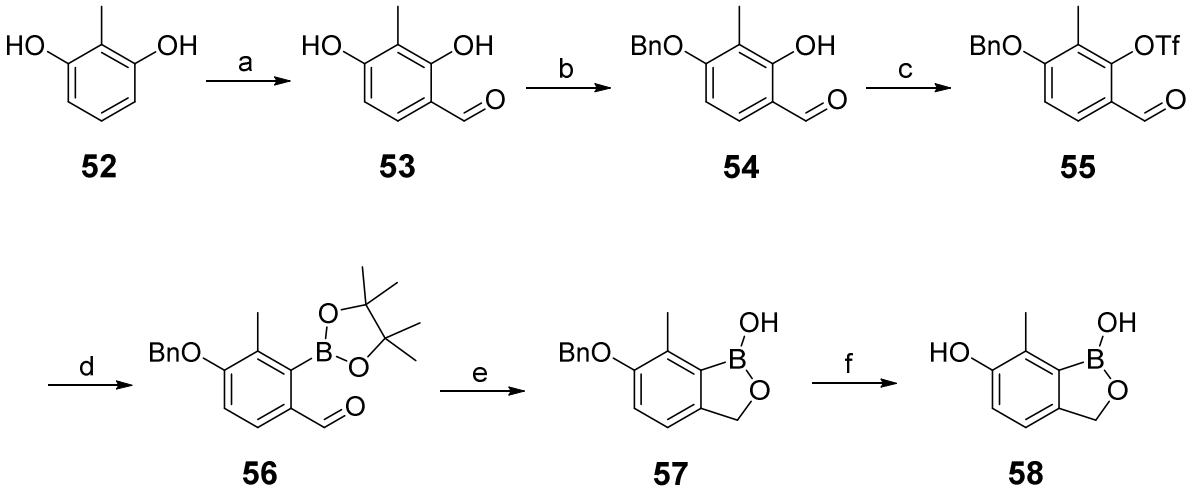
1: X = COOMe;
2: X = $CONH_2$



3: Y = $NHCH_2CH_2OMe$;
4: Y = $NHCH_2CH_2OH$;
5: Y = $NHCH_2CH(Me)OH$;
6: Y = $NHCH_2C(Me_2)OH$;
7: Y = $NHC(Me_2)CH_2OH$;
8: Y = $NHC(CH_2)_2CH_2OH$:
9: Y = $NHCH(Me)CH_2OH$:
10: Y = $NHCH(Me)CH_2OH$-(R);
11: Y = $NHCH(Me)CH_2OH$-(*S*);
12: Y = $NHCH(Et)CH_2OH$-(*R*);
13: Y = $NHCH(Et)CH_2OH$-(*S*);
14: Y = $NHCH(n\text{Pr})CH_2OH$-(*R*);
15: Y = $NHCH(n\text{Pr})CH_2OH$-(*S*);
16: Y = $NHCH(i\text{Pr})CH_2OH$-(*R*);
17: Y = $NHCH(i\text{Pr})CH_2OH$-(*S*);
18: Y = $NHCH(c\text{Pr})CH_2OH$-(*R*);
19: Y = $NHCH(c\text{Pr})CH_2OH$-(*S*);
20: Y = $NHCH(CH_2CF_3)CH_2OH$-(*R*);
21: Y = $NHCH(CH_2CF_3)CH_2OH$-(*S*);
22: Y = $NHCH(CH_2CH_2CF_3)CH_2OH$-(*R*);
23: Y = $NHCH(CH_2CH_2CF_3)CH_2OH$-(*S*);
24: Y = $NHCH(n\text{Bu})CH_2OH$-(*R*);
25: Y = $NHCH(Ph)CH_2OH$-(*R*);
26: Y = $NHCH(Bn)CH_2OH$-(*R*);
27: Y = $NHCH(CH_2OMe)CH_2OH$-(*S*);
28: Y = $NHCH(CH_2SMe)CH_2OH$-(*S*);
29: Y = $NHCH(CH_2SO_2Me)CH_2OH$-(*S*);
30: Y = NHCH(Me)CH(Me)OH;
31: Y = NH((1*S*,2*S*)-2-hydroxycyclopentyl);
32: Y = NH((1*R*,2*R*)-2-hydroxycyclopentyl);
33: Y = NH(*cis*-(2-hydroxycyclopentyl));
34: Y = $NHCH(Me)C(Me_2)OH$-(*S*);
35: Y = $NHCH(Me)C(Me_2)OH$-(*R*);
36: Y = $NHCH(Et)C(Me_2)OH$-(*S*);
37: Y = $NHCH(Et)C(Me_2)OH$-(*R*);
38: Y = $NHCH(n\text{Pr})C(Me_2)OH$-(*S*);
39: Y = $NHCH(n\text{Pr})C(Me_2)OH$-(*R*);
40: Y = $N(Me)CH_2CH_2OH$;
41: Y = $N(Me)CH(Pr)CH_2OH$-(*R*);
42: Y = $N(CH_2CH_2OH)_2$;
43: Y = (*S*)-2-(hydroxymethyl)piperazin-1-yl;
44: Y = (*R*)-2-(hydroxymethyl)piperazin-1-yl;
45: Y = 3-hydroxy-3-methylpyrrolidin-1-yl;
46: Y = 3-hydroxy-3-methylazetidin-1-yl;
47: Y = 3-hydroxy-3-ethylazetidin-1-yl;
48: Y = 3-hydroxy-3-propylazetidin-1-yl;
49: Y = 3-hydroxy-3-isopropylazetidin-1-yl;
50: Y = 3-hydroxy-3-cyclopropylazetidin-1-yl;
51: Y = 3-hydroxy-3-trifluoromethylazetidin-1-yl.

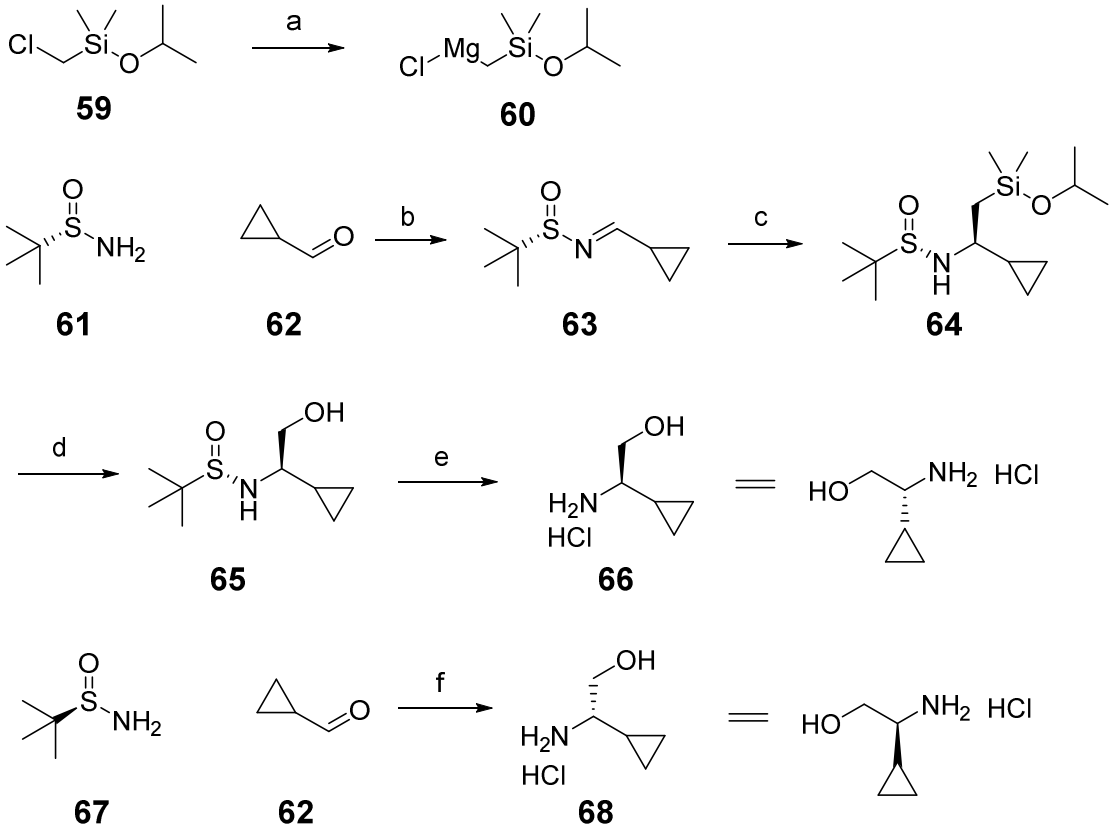
Figure 1. Building on previous antimalarial benzoxaborole molecules (**1** and **2**) [20], novel compounds (**3-51**) were designed and synthesized for a lead optimization program to improve potency, pharmacokinetic property and *in vivo* efficacy.

Scheme 1. Synthesis of a common benzoxaborole intermediate **58**



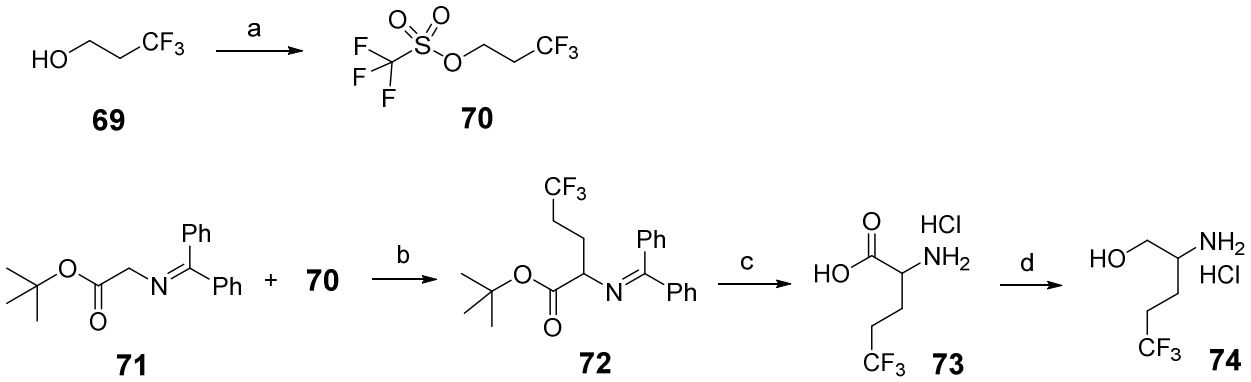
Reagents and conditions: (a) $POCl_3$, DMF, N_2, 0-20 ^oC, 16 h, then ice-water, 1 h; (b) BnBr, $NaHCO_3$, MeCN, 90 ^oC, 48 h; (c) Tf_2O, TEA, DCM, 0-20 ^oC, 4 h; (d) Pin_2B_2, $Pd(dppf)Cl_2$, 1,4-dioxane, N_2, 80 ^oC, 12 h; (e) $NaBH_4$, MeOH, THF, 5 ^oC to rt, 2h; (f) H_2, Pd/C, EtOAc, 50 psi, 25 ^oC, 12 h.

Scheme 2. Synthesis of 2-amino-2-alkylethanol intermediates **66** and **68**



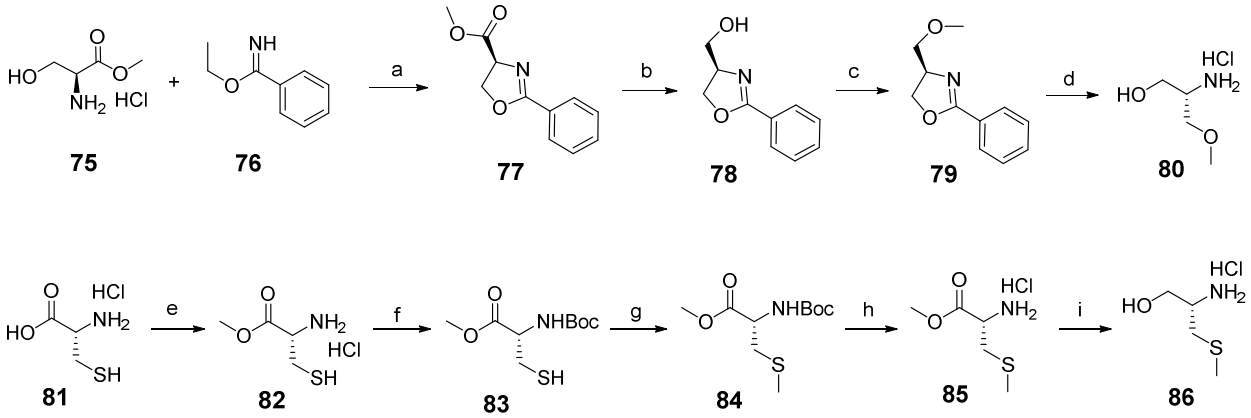
Reagents and conditions: (a) Mg, I_2, THF, N_2, reflux, 3 h; (b) $Ti(OEt)_4$, DCM, 50 ^oC, 16 h; (c) Grignard reagent **60**, THF, rt, 1 h; (d) $KHCO_3$, KF, H_2O_2, 45 ^oC, 2 h; (e) 4N HCl, MeOH, rt, 2 h; (f) Methods for **66** were used for **68**.

Scheme 3. Synthesis of 2-amino-2-alkylethanol intermediate **74**



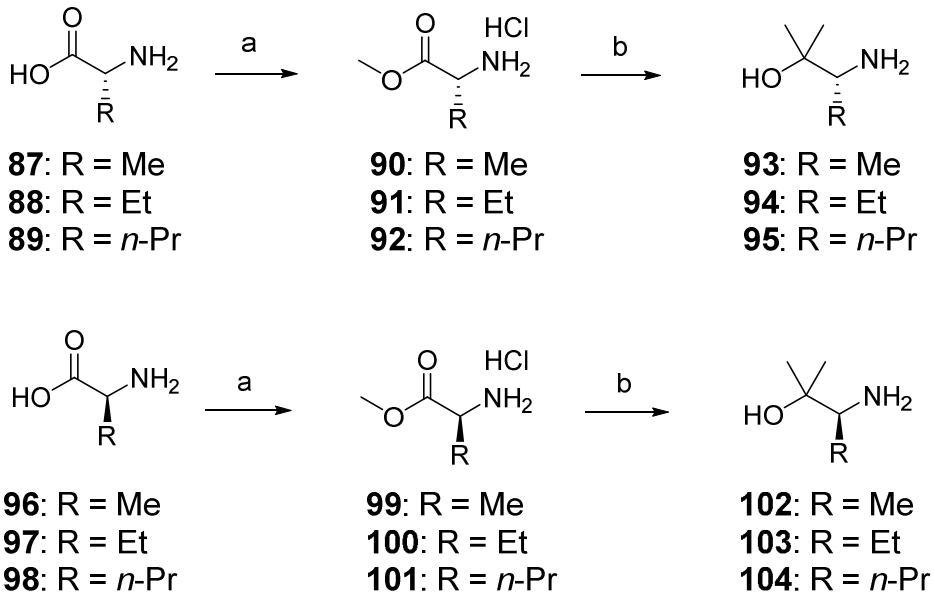
Reagents and conditions: (a) Tf_2O, 2,6-lutidine, DCM, -25 ^oC for 2 h and rt for 1 h; (b) LDA, THF, -78 ^oC to rt, 4 h; (c) 50% HCl, MeOH, reflux, 16 h; (d) LAH, THF, 70 ^oC for 16 h, work-up, then HCl, 1,4-dioxane, rt, 30 min.

Scheme 4. Synthesis of 2-amino-2-alkylethanol intermediates **80** and **86**



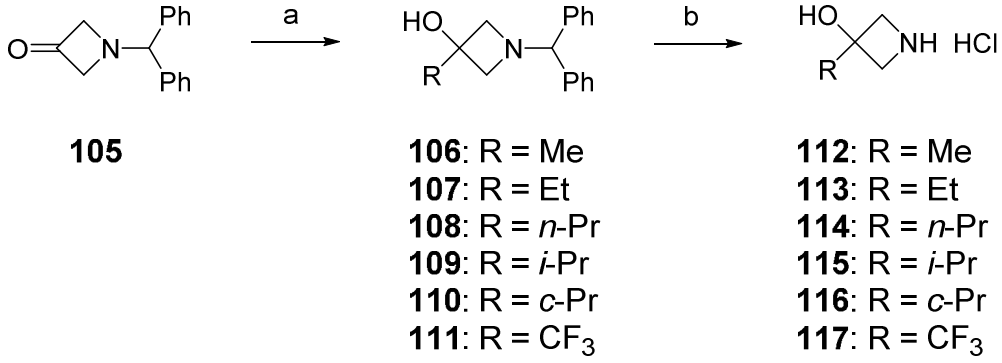
Reagents and conditions: (a) $ClCH_2CH_2Cl$, 84 ^oC, 16 h; (b) LAH, THF, 0 ^oC to 70 ^oC, 2 h; (c) NaH, MeI, 0 ^oC to rt, 16 h; (d) 4N HCl, reflux, 16 h; (e) $SOCl_2$, MeOH, 60 ^oC, 16 h; (f) Boc_2O, Et_3N, DCM, rt, 16 h; (g) MeI, DIPEA, DCM, rt, 16 h; (h) 4N HCl, 1,4-dioxane, rt, 16 h; (i) LAH, THF, 70 ^oC, then HCl, 1,4-dioxane.

Scheme 5. Synthesis of substituted 2-aminoethanol intermediates **93-95** and **102-104**



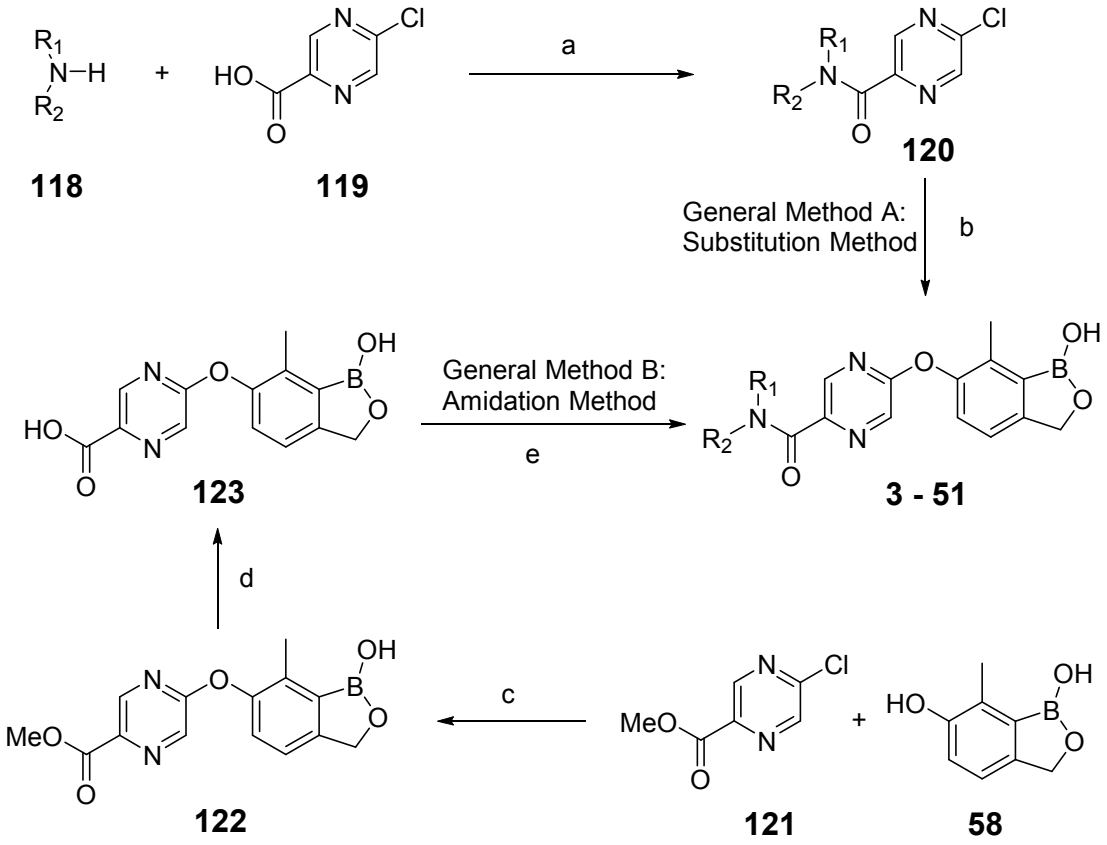
Reagents and conditions: (a) $SOCl_2$, MeOH, 0 ^oC to reflux, 2 h; (b) MeMgBr, THF, 0 ^oC to rt, 16 h.

Scheme 6. Synthesis of 3-alkylazetidin-3-ol intermediates **112-117**



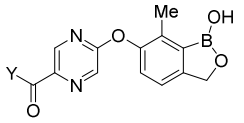
Reagents and conditions: (a) for intermediates **106-110**, RMgBr, THF, -78 ^oC for 2 h and rt; for **111**, Me_3SiCF_3, CsF, THF, rt, 1 h; (b) H_2, Pd/C, MeOH, rt, 16 h.

Scheme 7. Synthesis of compounds **3-51** using two general methods



Reagents and conditions: (a) four methods used to synthesize amides: (i) the carboxylic acid, $SOCl_2$, DCM, 50 °C, 16 h, then the corresponding amino alcohol, pyridine or TEA, 50 °C, 3 h; or (ii) HATU, DIPEA, DMF, rt, 1-2 h; or (iii) CMPI, DIPEA, NMP, rt, 16 h; or (iv) the corresponding acid, isobutyl chloroformate, TEA, DCM, 0 °C, 30 min, then the corresponding amino alcohol, 10 min; (b) **58**, Cs_2CO_3, DMF, 80 °C, 16 h; (c) Cs_2CO_3, DMF, rt, 16 h; (d) $LiOH \cdot H_2O$, MeOH and H_2O, rt, 16 h, then 1N HCl; (e) the corresponding amino alcohol, HATU, DIPEA, DMF, rt, 0.5-2 h.

Table 1. *In vitro* activity results for compounds **3-51** against cultured *P. falciparum*.[a]



Compound	Group Y	cLogD$_{7.4}$ [b]	tPSA [b]	IC$_{50}$ (μM, W2)	IC$_{50}$ (μM, 3D7)
3	NHCH$_2$CH$_2$OMe	0.83	102.8	0.788	0.644
4	NHCH$_2$CH$_2$OH	0.25	113.8	0.372	1.08
5	NHCH$_2$CH(Me)OH	0.66	113.8	1.28	1.1
6	NHCH$_2$C(Me$_2$)OH	0.74	113.8	0.334	0.554
7	NHC(Me$_2$)CH$_2$OH	0.74	113.8	0.29	0.425
8	NHC(CH$_2$)$_2$CH$_2$OH	0.38	113.8	2.01	1.5
9	NHCH(Me)CH$_2$OH	0.66	113.8	0.342	0.994
10	NHCH(Me)CH$_2$OH-(*R*)	0.66	113.8	0.944	0.848
11	NHCH(Me)CH$_2$OH-(*S*)	0.66	113.8	0.844	0.808
12	NHCH(Et)CH$_2$OH-(*R*)	1.13	113.8	0.136	0.403
13	NHCH(Et)CH$_2$OH-(*S*)	1.13	113.8	0.704	0.801
14	NHCH(*n*Pr)CH$_2$OH-(*R*)	1.53	113.8	0.161	0.201
15	NHCH(*n*Pr)CH$_2$OH-(*S*)	1.53	113.8	0.297	0.715
16	NHCH(*i*Pr)CH$_2$OH-(*R*)	1.54	113.8	0.149	0.466
17	NHCH(*i*Pr)CH$_2$OH-(*S*)	1.54	113.8	0.774	1.99
18	NHCH(*c*Pr)CH$_2$OH-(*R*)	1.03	113.8	0.492	0.587
19	NHCH(*c*Pr)CH$_2$OH-(*S*)	1.03	113.8	0.643	1.09
20	NHCH(CH$_2$CF$_3$)CH$_2$OH-(*R*)	1.54	113.8	0.0822	0.149
21	NHCH(CH$_2$CF$_3$)CH$_2$OH-(*S*)	1.54	113.8	0.35	0.031
22	NHCH(CH$_2$CH$_2$CF$_3$)CH$_2$OH-(*R*)	1.79	113.8	0.23	0.48
23	NHCH(CH$_2$CH$_2$CF$_3$)CH$_2$OH-(*S*)	1.79	113.8	0.352	0.959
24	NHCH(*n*Bu)CH$_2$OH-(*R*)	1.93	113.8	0.0849	0.215
25	NHCH(Ph)CH$_2$OH-(*R*)	2.10	113.8	0.103	0.161
26	NHCH(Bn)CH$_2$OH-(*R*)	2.35	113.8	0.115	0.505
27	NHCH(CH$_2$OMe)CH$_2$OH-(*S*)	0.16	123.0	0.455	0.796
28	NHCH(CH$_2$SMe)CH$_2$OH-(*S*)	0.99	113.8	0.037	0.074
29	NHCH(CH$_2$SO$_2$Me)CH$_2$OH-(*S*)	-0.97	147.9	0.18	0.472
30	NHCH(Me)CH(Me)OH	1.08	113.8	0.513	1.46
31	NH((*1S,2S*)-2-hydroxycyclopentyl)	1.11	113.8	0.149	0.537
32	NH((*1R,2R*)-2-hydroxycyclopentyl)	1.11	113.8	0.133	0.245
33	NH(*cis*-(2-hydroxycyclopentyl))	1.11	113.8	0.292	0.181
34	NHCH(Me)C(Me$_2$)OH-(*S*)	1.16	113.8	0.202	0.327
35	NHCH(Me)C(Me$_2$)OH-(*R*)	1.16	113.8	0.265	0.374
36	NHCH(Et)C(Me$_2$)OH-(*S*)	1.62	113.8	0.279	0.829
37	NHCH(Et)C(Me$_2$)OH-(*R*)	1.62	113.8	0.0918	0.222
38	NHCH(*n*Pr)C(Me$_2$)OH-(*S*)	2.02	113.8	0.129	0.217

39	NHCH(*n*Pr)C(Me_2)OH-(*R*)	2.02	113.8	0.26	0.553
40	N(Me)CH_2CH_2OH	0.50	105.0	0.262	0.437
41	N(Me)CH(Pr)CH_2OH-(*R*)	1.78	105.0	0.0481	0.252
42	N($CH_2CH_2OH)_2$	-0.25	125.2	0.328	0.184
43	(*S*)-2-(hydroxymethyl)piperazin-1-yl	-0.29	117.0	0.0982	0.171
44	(*R*)-2-(hydroxymethyl)piperazin-1-yl	-0.29	117.0	0.103	0.151
45	3-hydroxy-3-methylpyrrolidin-1-yl	0.52	105.0	0.139	0.314
46	3-hydroxy-3-methylazetidin-1-yl	0.47	105.0	0.0326	0.043
47	3-hydroxy-3-ethylazetidin-1-yl	0.94	105.0	0.0289	0.055
48	3-hydroxy-3-propylazetidin-1-yl	1.33	105.0	0.0159	0.054
49	3-hydroxy-3-isopropylazetidin-1-yl	1.34	105.0	0.0407	0.057
50	3-hydroxy-3-cyclopropylazetidin-1-yl	0.84	105.0	0.0517	0.134
51	3-hydroxy-3-trifluoromethylazetidin-1-yl	1.10	105.0	0.0066	0.008
artemisinin		3.11	54.0	0.006	na [c]
chloroquine		0.88	28.2	0.021	na [c]
atovaquone		3.33	54.4	na [c]	0.001

[a] Experimental procedures for the *in vitro* assays for the W2 and 3D7 strains were reported previously.[20] IC_{50} values were determined from two independent replicates for W2 strain and three replicates for 3D7 strain. 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. [b] $cLogD_{7.4}$ and tPSA values were calculated using ChemAxon software. [c] Not applicable.

Table 2. Pharmacokinetic parameters for **4**, **9**, **14**, **20** and **46** in mouse, rat or dog [a]

Compound	$cLogD_{7.4}$	oral PK parameters					intravenous PK parameters			
		C_{max} (ug/mL)	T_{max} (h)	$AUC_{0\text{-}inf}$ (h*ug/mL)	$T_{1/2}$ (h)	*F* (%)	CL (mL/h/kg)	V_{ss} (mL/kg)	$AUC_{0\text{-}inf}$ (h*ug/mL)	$T_{1/2}$ (h)
4 [b]	0.25	4.3	1.0	29.6	3.0	94	159	636	31.4	3.0
9 [b]	0.66	3.8	1.0	33.0	2.7	85	128	603	39.0	3.1
14 [b]	1.53	4.0	0.5	21.6	4.5	103	243	874	20.5	4.2
20 [b]	1.54	2.1	1.0	13.4	3.0	64	238	741	21.0	3.0
46 [b]	0.47	3.9	0.5	20.7	6.8	101	244	888	20.5	7.1
46 [c]	0.47	1.7	3.5	20.8	5.2	96	233	831	21.7	4.6
46 [d]	0.47	112	1.2	214	9.5	127	30.3	290	168	6.9

[a] Abbreviations: C_{max}, maximum concentration of drug in plasma; T_{max}, time to maximum concentration of drug in plasma; $AUC_{0\text{-}inf}$, 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] Mice dosed at 5 mg/kg orally or intravenously; [c] Rats dosed at 5 mg/kg orally or intravenously; [d] Dogs dosed at 5 mg/kg orally or intravenously.

Table 3. Percent survival of mice and efficacies of **4**, **9**, **12**, **14**, **16**, **20**, **46**, **47**, **48**, **49, 50** and **51** in a *P. berghei* mouse malaria model.[a]

Compound	Oral dosage	Percent survival of mice on specific days (%)														ED_{90} [c] (mg/kg)
		d4 [b]	d5	d6	d7	d8	d9	d10	d11	d12	d13	d14	d15	d16-d19	d20	
4	200mg/kg	100	100	100	100	80	80	0								56.1
	100 mg/kg	100	100	100	80	40	40	0								
	33 mg/kg	100	100	100	0											
	11 mg/kg	100	100	100	0											
	3.7 mg/kg	100	100	100	0											
	1.2 mg/kg	100	100	100	0											
9	100 mg/kg	100	100	100	100	100	80	40	40	0						44.3
	33 mg/kg	100	100	100	100	0										
	11 mg/kg	100	40	40	40	0										
	3 mg/kg	100	60	60	60	0										
	1 mg/kg	100	20	20	20	0										
	0.3 mg/kg	100	20	20	20	0										
12	200 mg/kg	100	100	100	100	100	60	20	0							43.3
	100 mg/kg	100	100	100	100	80	0									
	33 mg/kg	100	100	100	0											
	11 mg/kg	100	100	100	0											
	3 mg/kg	100	100	100	0											
	1 mg/kg	100	100	100	0											
14	200 mg/kg	100	100	100	100	100	100	100	100	100	100	100	100	100	100	11.7
	100 mg/kg	100	100	100	100	100	100	100	20	20	20	0				
	33 mg/kg	100	100	100	100	100	0									
	11 mg/kg	100	100	100	0											
	3.7 mg/kg	100	100	100	0											
	1.2 mg/kg	100	100	100	0											
16	100 mg/kg	100	100	100	100	100	100	40	40	40	40	40				26.7
	33 mg/kg	100	100	100	80	80	0									
	11 mg/kg	100	100	100	0											
	3 mg/kg	100	100	100	0											
	1 mg/kg	100	100	100	0											
20	100 mg/kg	100	100	100	100	100	100	100	0							7.3
	33 mg/kg	100	100	100	100	100	100	60	0							
	11 mg/kg	100	100	100	0											
	3.7 mg/kg	100	100	100	0											
	1.2 mg/kg	100	100	100	0											
	0.4 mg/kg	100	100	100	0											
46	100 mg/kg	100	100	100	100	100	100	100	100	100	100	60	60	60-0 [d]		1.9
	33 mg/kg	100	100	100	100	100	100	100	100	100	100	0				
	11 mg/kg	100	100	100	100	100	0									
	3.7 mg/kg	100	100	100	60	0										
	1.2 mg/kg	100	100	100	0											
	0.4 mg/kg	100	100	100	0											
	0.1 mg/kg	100	100	100	0											
47	200 mg/kg	100	100	100	100	100	100	100	100	100	100	100	100	80	80	4.8
	100 mg/kg	100	100	100	100	100	100	100	100	100	100	0				
	33 mg/kg	100	100	100	100	100	80	20	20	20	20	0				
	11 mg/kg	100	100	100	40	0										
	3.7 mg/kg	100	100	100	0											
	1.2 mg/kg	100	100	100	0											
	0.4 mg/kg	100	100	100	0											
	0.1 mg/kg	100	100	100	0											

48	200 mg/kg	100	100	100	100	100	100	100	100	100	100	100	100	100-0 [e]	0	3.3
	100 mg/kg	100	100	100	100	100	100	100	100	100	100	80	80	20	0	
	33 mg/kg	100	100	100	100	100	100	100	100	100	100	0				
	11 mg/kg	100	100	100	80	80	0									
	3.7 mg/kg	100	100	100	0											
	1.2 mg/kg	100	100	100	0											
	0.4 mg/kg	100	100	100	0											
	0.1 mg/kg	100	100	100	0											
49	200 mg/kg	100	100	100	100	100	100	100	100	100	100	100	100	100	100	4.5
	100 mg/kg	100	100	100	100	100	100	100	100	100	100	100	100	100-80 [f]	80	
	33 mg/kg	100	100	100	100	100	100	100	100	100	100	0				
	11 mg/kg	100	100	100	60	60	60	20	20	20	20	0				
	3.7 mg/kg	100	100	100	0											
	1.1 mg/kg	100	100	100	0											
	0.4 mg/kg	100	100	100	0											
	0.1 mg/kg	100	100	100	0											
50	100 mg/kg	100	100	100	100	100	100	100	100	100	100	0				10.3
	33 mg/kg	100	100	100	100	100	100	40	40	40	40	0				
	11 mg/kg	100	100	100	0											
	3.7 mg/kg	100	100	100	0											
	1.2 mg/kg	100	100	100	0											
	0.4 mg/kg	100	100	100	0											
	0.1 mg/kg	100	100	100	0											
51	100 mg/kg	100	100	100	100	100	100	100	100	100	100	100	100	100	100	5.7
	33 mg/kg	100	100	100	100	100	100	50	50	50	50	0				
	11 mg/kg	100	100	100	100	0										
	3.7 mg/kg	100	100	100	0											
	1.1 mg/kg	100	100	100	0											
	0.4 mg/kg	100	100	100	0											
	0.1 mg/kg	100	100	100	0											
	CQ 30 mg/kg [g]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	NA [i]
	Vehicle [h]	100	0													NA

[a] The model evaluated the therapeutic efficacy of a molecule against *Plasmodium berghei*. Mice were infected by the intraperitoneal injection of *Plasmodium berghei*-infected erythrocytes collected from a previously infected animal. 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] 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; [d] 60% on day 16 and 0% on day 17; [e] 100% on day 16 and 0% on day 17; [f] 100% on days 16 & 17 and 80% on days 18 & 19. [g] Because of the nature of the *in vivo* model, there was efficacy variation of the chloroquine positive control group and 100% survival was commonly observed on day 20 on average; [h] Because of the nature of the *in vivo* model, there was slight variation of the vehicle control group and 100% survival was observed on days 4 to 6. [i] NA = not applicable.

Table 4. Efficacy results of **14**, **46**, **47**, **49**, **50** and **51** in a *P. falciparum* mouse malaria model.[a]

Compound	$cLogD_{7.4}$ [b]	ED_{90} (mg/kg) [c]	AUC_{ED90} (ug·h/mL/day) [d]
14	1.53	2.5	7.45
46	0.47	0.85	1.4
47	0.94	0.84	1.6
49	1.34	<5	ND [e]
50	0.84	<5	ND [e]
51	1.10	<5	ND [e]

[a] The model evaluated the therapeutic efficacy of a molecule against *Plasmodium falciparum Pf*3D7$^{0087/N9}$ growing in peripheral blood of NODscidIL2Rγ^{null} mice engrafted with human erythrocytes. 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] $cLogD_{7.4}$ values were calculated using ChemAxon software; [c] ED_{90} values were based on comparisons of parasitemias with those of mice treated only with vehicle on day 4 after the onset of therapy; [d] AUC_{ED90} was the compound exposure in whole blood necessary to reduce *P. falciparum* parasitemia in peripheral blood at day 7 after infection by 90% with respect to vehicle-treated mice. [e] Not determined.

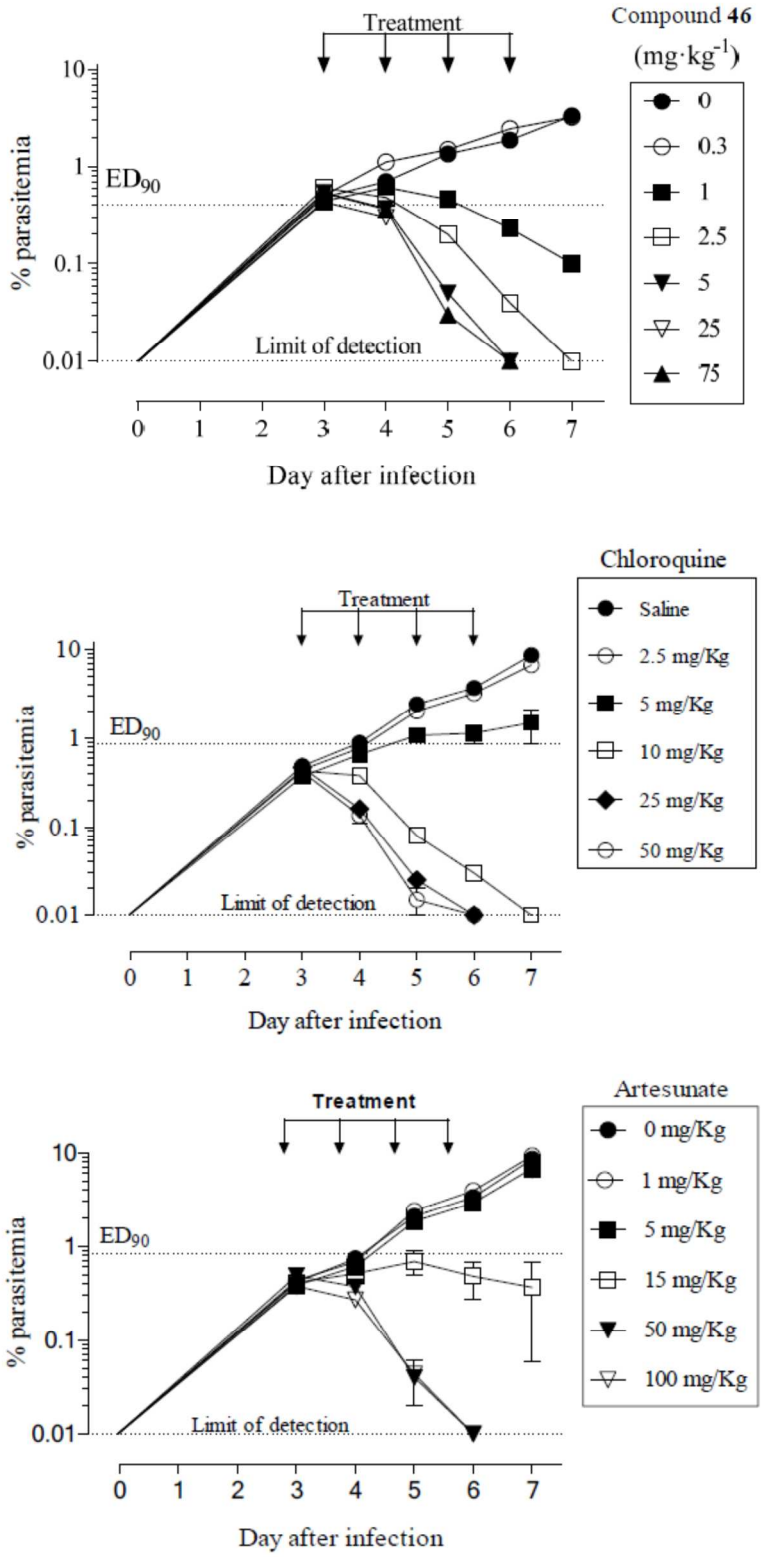


Figure 2. Compound **46** Demonstrated rapid *in vivo* parasite clearance in *P. falciparum*-infected mouse model, similar to artesunate and chloroquine of two well-known fast parasite-killing antimalarial agents.

Table 5. *In vitro* activities of **46** against *P. falciparum* parasite strains [a]

Compound	IC_{50} (μM) [b]										
	NF-54	K1	T9/94	HB3	TM90C 2A	TM90C 2B	Dd2	V1/S	7G8	D6	FCB
46	0.036	0.062	0.054	0.08	0.064	0.06	0.053	0.063	0.035	0.062	0.066
Atovaquone	0.001	0.0007	0.001	0	0.001	> 0.035	0.002	0.001	ND [c]	ND	ND
Chloroquine	0.02	0.48	0.384	0.027	0.384	0.561	0.69	> 1	ND	ND	ND
Pyrimethamine	0.045	> 20	0.038	3.381	> 20	> 20	> 20	> 20	ND	ND	ND

[a] Experimental procedure for the *in vitro* assay was same as that reported previously for IC_{50} determination against *P.f.* 3D7 strain;[20] [b] NF-54, K1, T9/94, HB3, TM90C-2A, TM90C-2B, Dd2, V1/S, 7G8, D6 and FCB are *P. falciparum* parasite strains; [c] Not determined.

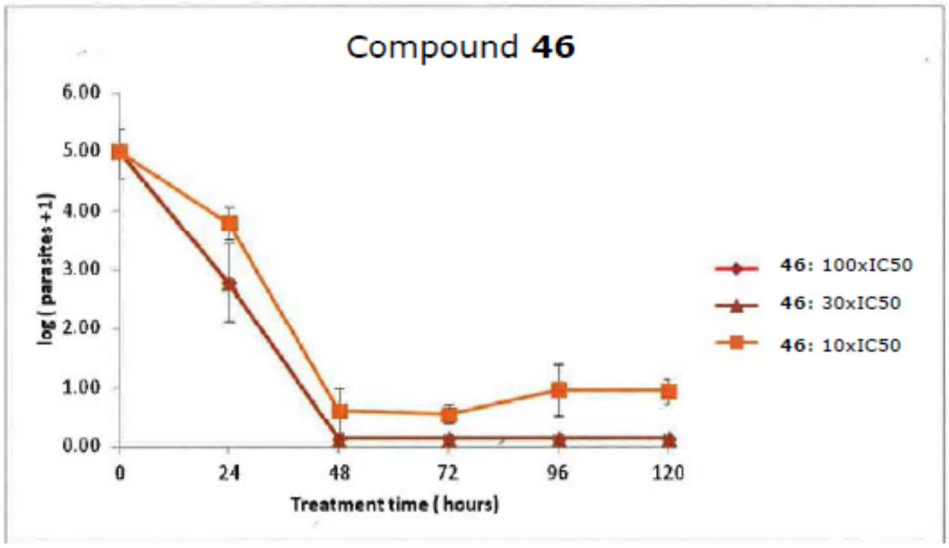


Figure 3. Parasite-killing curves of compound **46** at 10×, 30× and 100×IC_{50} doses indicating that the concentration required for **46** to achieve a maximal rate of parasite-killing was 10× to 30×IC_{50}.

Table 6. Parasite reduction ratio (PRR) parameters of compound **46** in comparison with four standard antimalarial agents [a]

Compound	Dose	Lag Phase (h) [b]	slope	r	Log PRR	$PCT_{99.9\%}$ (h) [c]
46	$10 \times IC_{50}$	0	-0.0913	-0.936	4.1	36
	$30 \times IC_{50}$	0	-0.1015	-0.998	4.9	30
	$100 \times IC_{50}$	0	-0.1015	-0.998	4.9	30
Artemisinin	$10 \times IC_{50}$	0	-0.101	-0.920	>4.8	<24
Atovaquone	$10 \times IC_{50}$	48	-0.061	-0.992	2.9	81
Pyrimethamine	$10 \times IC_{50}$	24	-0.061	-0.994	2.9	61
chloroquine	$10 \times IC_{50}$	0	-0.094	-0.999	4.5	34

[a] The killing curve data were analyzed to fit to the linear stretch to determine the PRR parameters indicating that compound **46** antiparasitic action was fast and similar to artemisinin and chloroquine. PRR represents parasite reduction ratio or number of parasites the compound could kill in a parasite life cycle; [b] Lag phase was the time needed to show the maximal killing effects of the molecule; [c] $PCT_{99.9\%}$ was the parasite clearance time to kill 99.9% of the initial parasite population.

Table 7. Results of the bacterial reverse mutation assay (Ames assay) of compound **46** [a]

Dose (μg/plate)	Average revertants/plate [b]									
	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
	without activation	with activation [c]	without activation	with activation	without activation	with activation	without activation	with activation	without activation	with activation
vehicle [d]	14 ± 6	19 ± 8	154 ± 9	141 ± 1	10 ± 3	10 ± 0	19 ± 9	11 ± 4	45 ± 9	42 ± 6
1.5 [e]	15 ± 7	20 ± 6	144 ± 6	150 ± 17	12 ± 3	9 ± 2	15 ± 3	12 ± 0	40 ± 1	39 ± 8
5.0	12 ± 4	15 ± 1	152 ± 1	128 ± 5	8 ± 1	16 ± 8	15 ± 2	22 ± 6	35 ± 6	37 ± 8
15	15 ± 8	13 ± 4	126 ± 15	149 ± 24	13 ± 1	12 ± 6	10 ± 3	9 ± 0	41 ± 8	50 ± 12
50	14 ± 3	17 ± 5	132 ± 0	136 ± 17	8 ± 6	11 ± 3	12 ± 4	9 ± 2	35 ± 14	44 ± 18
150	13 ± 8	10 ± 4	148 ± 6	154 ± 3	8 ± 1	15 ± 1	15 ± 4	12 ± 7	56 ± 6	53 ± 21
500	10 ± 0	18 ± 5	155 ± 4	132 ± 5	5 ± 0	6 ± 4	14 ± 12	17 ± 5	55 ± 11	35 ± 18
1500	13 ± 0	15 ± 4	133 ± 8	144 ± 5	7 ± 4	10 ± 4	10 ± 1	8 ± 4	33 ± 4	26 ± 1
5000	9 ± 1	18 ± 3	20 ± 13	31 ± 16	4 ± 1	6 ± 2	10 ± 4	9 ± 5	43 ± 3	34 ± 1
positive [f]	162 ± 15	524 ± 42	484 ± 21	550 ± 47	356 ± 43	163 ± 21	430 ± 199	82 ± 2	357 ± 81	311 ± 39

[a] Testing strains were *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvr*A. [b] The data demonstrated that compound **46** was not mutagenic with or without metabolic activation under the conditions tested. [c] Rat liver S9 was used for the metabolic activation condition. [d] Vehicle (DMSO) was used as the negative control. [e] The test article was prepared in DMSO and tested via the plate incorporation method at eight dose levels as shown in the same column of this table. [f] Positive controls were 2-nitrofluorene (1.0 μg/plate) for TA98 without activation, 2-aminoanthracene (2.0 μg/plate) for TA98 or TA100 or TA1535 or TA1537 with activation, sodium azide (1.0 μg/plate) for TA100 or TA1535 without activation, 9-aminoacridine (75 μg/plate) for TA1537 without activation, methyl methanesulfonate (1000 μg/plate) for WP2 *uvr*A without activation, and 2-aminoanthracene (15.0 μg/plate) for WP2 *uvr*A with activation.

Table 8. Results of definitive assay for micronucleus induction *in vitro* in human lymphocytes by compound **46** [a]

Assay group	Treatment	Replication Index [b]	Cytotoxicity (%) [c]	%Micronucleated Cells	Mean %Micronucleated Cells
3-Hour Treatment with S9	DMSO (1%)	0.83	0	0.76	0.63
		0.86		0.50	
	MMC (0.33 µg/mL)	ND [d]	ND	3.52	2.98
				2.40	
	46 (80 µg/mL)	0.83	1	0.88	0.74
		0.84		0.60	
	46 (160 µg/mL)	0.77	8	0.39	0.50
		0.79		0.60	
	46 (355.15 µg/mL)	0.82	7	0.70	0.73
		0.75		0.75	
3-Hour Treatment without S9	DMSO (1%)	0.84	0	1.18	0.99
		0.89		0.80	
	MMC (0.84 µg/mL)	ND	ND	7.16	5.26
				3.40	
	46 (80 µg/mL)	0.86	3	0.50	0.81
		0.82		1.11	
	46 (160 µg/mL)	0.87	2	1.22	1.18
		0.83		1.15	
	46 (355.15 µg/mL)	0.78	7	0.68	0.85
		0.83		0.99	
28-Hour Treatment without S9	DMSO (1%)	0.84	0	0.60	0.74
		0.87		0.89	
	MMC (0.33 µg/mL)	ND	ND	6.60	6.76
				6.92	
	46 (40 µg/mL)	0.78	7	1.18	1.18
		0.81		1.19	
	46 (80 µg/mL)	0.79	7	1.07	1.28
		0.80		1.49	
	46 (160 µg/mL)	0.75	13	0.73	0.89
		0.74		1.07	
	46 (355.15 µg/mL)	0.58	33	0.89	0.95
		0.57		0.99	

[a] MMC (Mitomycin C) was the positive control. At least 1000 binucleated cells scored for micronuclei per culture. No statistically significant increase in the frequency of micronucleus-binucleus cells in the compound **46** treated group was observed at any concentration relative to the concurrent solvent control group ($p > 0.05$ by Fisher's Exact test), and no dose-related response was observed either; [b] Replication Index (RI) = (number of binucleated cells + 2 × number of multinucleated cells) / total number of cells; [c] Cytotoxicity (%) = 100 – (RI of treated culture / RI of vehicle control) × 100; [d] ND = Not determined.

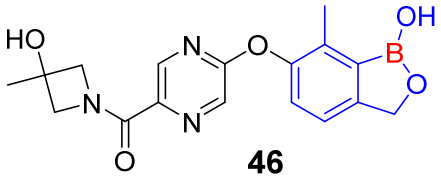
Table 9: Bone marrow micronucleus analysis from the *in vivo* rat micronucleus study of **46** [a]

Treatment	Rat Gender	Time (h)	Animal Number	%PCE [b] (Mean ± SD)	Change from Control (%)	%MnPCE [c] (Mean ± SD)	Number of MnPCE/20000 PCEs Scored
Vehicle 0 mg/kg	M	24	5	46.9 ± 3.6	0	0.07 ± 0.05	14
46 500 mg/kg	M	24	5	45.0 ± 7.0	-4	0.03 ± 0.04	6
46 1000 mg/kg	M	24	5	46.7 ± 6.1	0	0.08 ± 0.04	15
46 2000 mg/kg	M	24	5	45.2 ± 7.0	-4	0.09 ± 0.03	18
CP 40 mg/kg	M	24	5	40.1 ± 2.2*	-14	3.19 ± 0.44**	638
Vehicle 0 mg/kg	M	48	5	42.5 ± 5.1	0	0.10 ± 0.01	19
46 2000 mg/kg	M	48	5	37.5 ± 4.7	-12	0.10 ± 0.03	19

[a] Route of administration was oral (PO); *p < 0.05 or **p < 0.01 using one-way ANOVA with post-hoc analysis or T-test; Positive control article was cyclophosphamide monohydrate (CP); Bone marrow collection time was 24 or 48 hours post-dose; Vehicle = 1% CMC (medium viscosity) in deionized water. Compound **46** was found not to have a micronucleus risk at all three doses tested, and positive and negative control values were found as expected; [b] PCE stands for polychromatic erythrocyte cells; [c] MnPCE stands for micronucleated polychromatic erythrocyte cells.

Graphic for table of contents only:

IC_{50} = 33 nM (*P.f.* W2 strain)
43 nM (*P.f.* 3D7 strain)
ED_{90} = 0.85 mg/kg in *P. f.* - infected mice
1.92 mg/kg in *P. b.* - infected mice



46