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Reverse Fosmidomycin Derivatives against the Antimalarial Drug Target IspC (Dxr)[†,‡]

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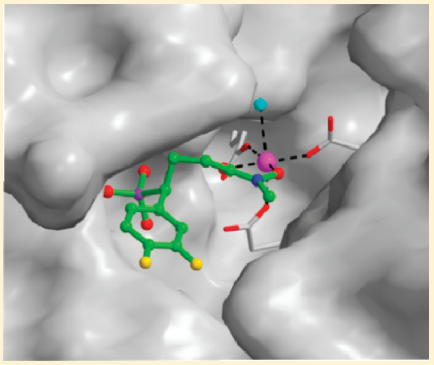
Supporting Information

ABSTRACT: Reverse hydroxamate-based inhibitors of IspC, a key enzyme of the non-mevalonate pathway of isoprenoid biosynthesis and a validated antimalarial target, were synthesized and biologically evaluated. The binding mode of one derivative in complex with *Ec*IspC and a divalent metal ion was clarified by X-ray analysis. Pilot experiments have demonstrated in vivo potential.



INTRODUCTION

Each year, malaria causes several hundred million infections resulting in approximately one million fatalities.[1] All currently used antimalarial drugs are subject to rapidly progressing attrition by resistance development.[2] The urgent need for novel therapeutic agents is universally acknowledged, but the resources for their development are still sadly limited.

IspC (Dxr) protein of *Plasmodium falciparum* is a clinically validated antimalarial target.[3] The antibiotic fosmidomycin (**1**), which acts as a slow-binding IspC inhibitor,[4] has been used successfully in clinical malaria trials.[5] However, its therapeutic use is hampered by the requirement for large and frequent doses, due to its unsatisfactory pharmacokinetic properties.[6]

IspC catalyzes the first committed step of the non-mevalonate pathway,[7] supplying essential isoprenoids in apicomplexan protozoa including *Plasmodium* but not in mammals that use the mevalonate pathway for that purpose.[8] Specifically, IspC converts a carbohydrate (1-deoxy-D-xylulose 5-phosphate, **2**) into a branched polyol (2*C*-methyl-D-erythritol 4-phosphate, **4**) by a skeletal rearrangement via **3** followed by hydride transfer.[9] The enzyme requires NADPH and a divalent cation as cofactors (Scheme 1).

1, initially isolated from *Streptomyces lavendulae*, resembles the structure of the IspC substrate but is endowed with a metabolically stable phosphonate group and a hydroxamic acid motif that is perfectly suited for formation of a chelate complex with the essential divalent metal ion of the enzyme.[10] Derivatives of **1** have been synthesized with the intention to provide drug candidates with improved pharmacokinetic and pharmacodynamic properties.[11] We now report on the synthesis and antiplasmodial activity of novel reverse fosmidomycin analogues. In addition, we provide kinetic and crystallographic evidence for their mode of action.

RESULTS AND DISCUSSION

Chemistry. C-Alkylation of starting materials **5a**−**d** with 2-(2-bromoethyl)-1,3-dioxolane in the presence of *n*-butyllithium provided 1,3-dioxolanes **6a**−**d** in good yields of 60−92%. Acidic hydrolysis of **6a**−**d** afforded the corresponding aldehydes **7a**−**d**, which were converted into carboxylic acids **8a**−**d** by oxidation with SeO_2 and H_2O_2.[12] The synthesis of *O*-benzyl-protected hydroxamic acids **9a,b**, **10a**−**c**, and **11** was accomplished by

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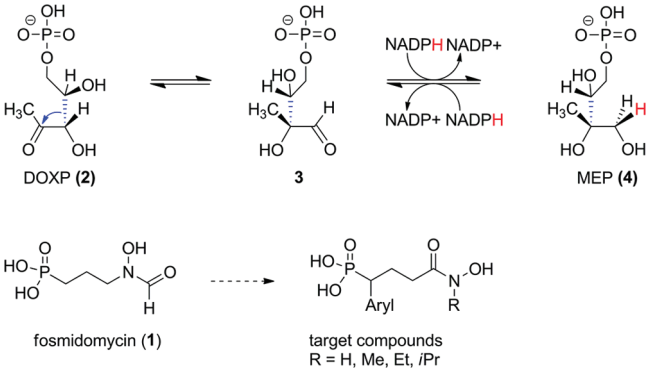
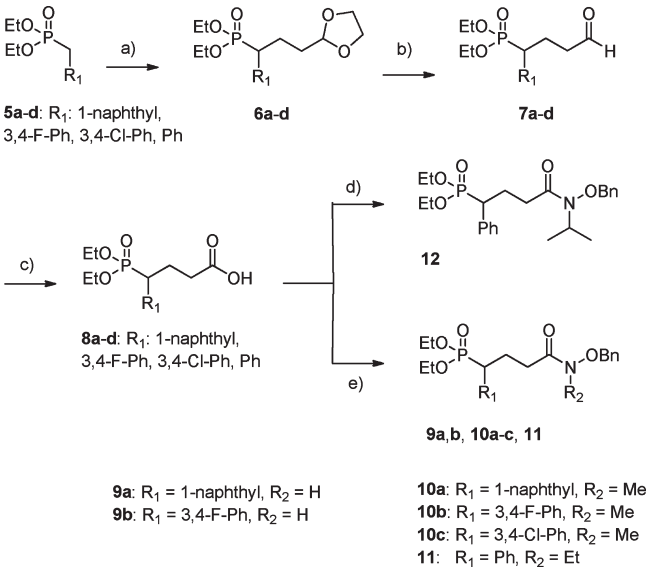
Scheme 1. Target Compounds and IspC-Catalyzed Reaction



Scheme 2. Synthesis of Intermediates 9−12[a]



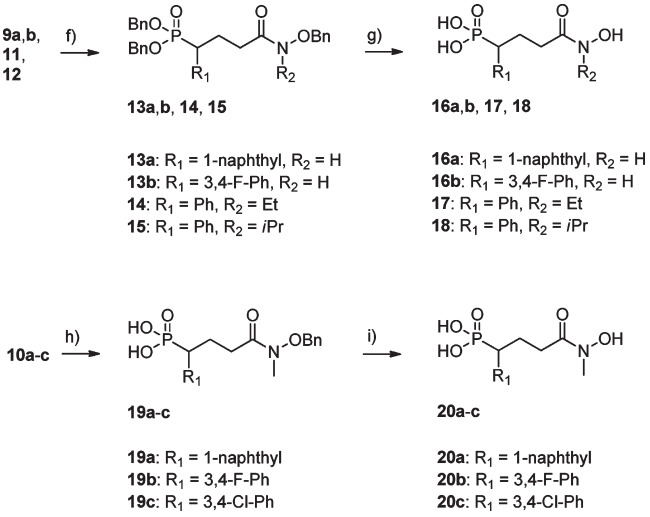
[a] Reagents and conditions: (a) *n*-BuLi, 2-(2-bromoethyl)-1,3-dioxolane, toluene, −78 °C; (b) HCl, acetone, 50 °C, 3 h; (c) SeO_2, H_2O_2, THF, 65 °C, 4 h; (d) 1,1′-carbonyldiimidazole, $BnONHR_2$ (R_2 = H, Me, Et), CH_2Cl_2, RT; (e) $C_2O_2Cl_2$/DMF, BnONH-*i*-Pr, RT.

1,1′-carbonyldiimidazole-mediated coupling reactions of carboxylic acids **8a**−**d** with *O*-benzylhydroxylamine, *N*-methyl-*O*-benzylhydroxylamine, and *N*-ethyl-*O*-benzylhydroxylamine, respectively.[13] However, the acylation of *N*-isopropyl-*O*-benzylhydroxylamine under similar reaction conditions failed. Finally, the *N*-isopropyl-substituted hydroxamic acid **12** was accessible in 81% yield by subsequent treatment of **8d** with oxalyl chloride, DMF, and *N*-isopropyl-*O*-benzylhydroxylamine (Scheme 2).

Next, we studied the two-step deprotection of *N*-methyl-substituted benzyloxyamides **10a**−**c**. Their reactions with bromotrimethylsilane (TMSBr) afforded crystalline phosphonic acids **19a**−**c** in good yields and high purity.[14]

Catalytic hydrogenation of **19a**−**c** finally yielded the required *N*-methyl-substituted hydroxamic acids **20a**−**c** in 87−98% yield. In contrast to the smooth dealkylation of substrates **10a**−**c**, the cleavage of ethyl phosphonates **9a**,**b**, **11**, and **12** in the presence of TMSBr was incomplete, and only crude product mixtures were obtained. However, transesterification of diethyl esters **9a**,**b**, **11**, and **12** provided *O*-benzyl protected compounds **13a**,**b**, **14**, and **15**, which were deprotected by catalytic hydrogenation to afford target compounds **16a**,**b**, **17**, and **18** (Scheme 3).[15]

Scheme 3. Synthesis of Target Compounds 16−20[a]



[a] Reagents and conditions: (f) (1) TMSBr, CH_2Cl_2, RT, 24 h; (2) THF/H_2O, RT, 1 h; (3) DCC, BnOH, benzene, 80 °C, 4 h; (g) H_2, Pd−C, MeOH, 3 h; (h) (1) TMSBr, CH_2Cl_2, RT, 24 h; (2) THF/H_2O, RT, 1 h; (i) H_2, Pd−C, MeOH, 1 h.

Table 1. In Vitro Inhibition Potency[a] and Cytotoxicity[c] of Target Compounds

	IC$_{50}$ (μM)			
compd	*Pf*IspC[a]	*Ec*IspC[a]	*Pf*K1[a,b]	MRC-5[c]
1[d]	0.14 ± 0.02	0.22 ± 0.01	3.7 ± 2.5	>62
16a	0.037 ± 0.003	7.4 ± 0.2	2.4 ± 1.1	>64
16b	0.003 ± 0.001	0.21 ± 0.02	0.38 ± 0.17	>64
17	0.015 ± 0.002	15 ± 0.4	1.3± 1.5	>64
18	inactive	inactive	inactive	>64
20a	0.009 ± 0.001	3.8 ± 0.2	0.97 ± 0.79	>62
20b	0.003 ± 0.001	0.12 ± 0.07	0.29 ± 0.20	>64
20c	0.004 ± 0.001	0.18 ± 0.02	0.41 ± 0.25	>64

[a] Enzyme assay. Values are the mean ± SD of three or more independent experiments. [b] Replication of *P. falciparum* in human erythrocytes. [c] Cytotoxicity test with MRC-5 cells. [d] IC$_{50}$ value according to ref 11a.

Biological Evaluation. As shown in Table 1, several compounds under study inhibit IspC protein of *P. falciparum* (*Pf*IspC) with IC$_{50}$ values in the single-digit nanomolar range, whereas the IC$_{50}$ values for the *Escherichia coli* enzyme (*Ec*IspC) exceed those for the *P. falciparum* enzyme by 1−3 orders of magnitude.

Some of the compounds studied are strong inhibitors of *P. falciparum* replication in erythrocytes in vitro, with IC$_{50}$ values in the low micromolar range (Table 1, column 4); notably, compounds **16b**, **20b**, and **20c** have about 10-fold higher potency than **1** against the chloroquine-resistant K1 strain of *P. falciparum*. Moreover, their toxicity in cell culture experiments is low, with IC$_{50}$ values above 60 μM (Table 1, column 5).

In summary, the in vitro evaluation has demonstrated that not only free but also *N*-methyl-substituted hydroxamic acids are

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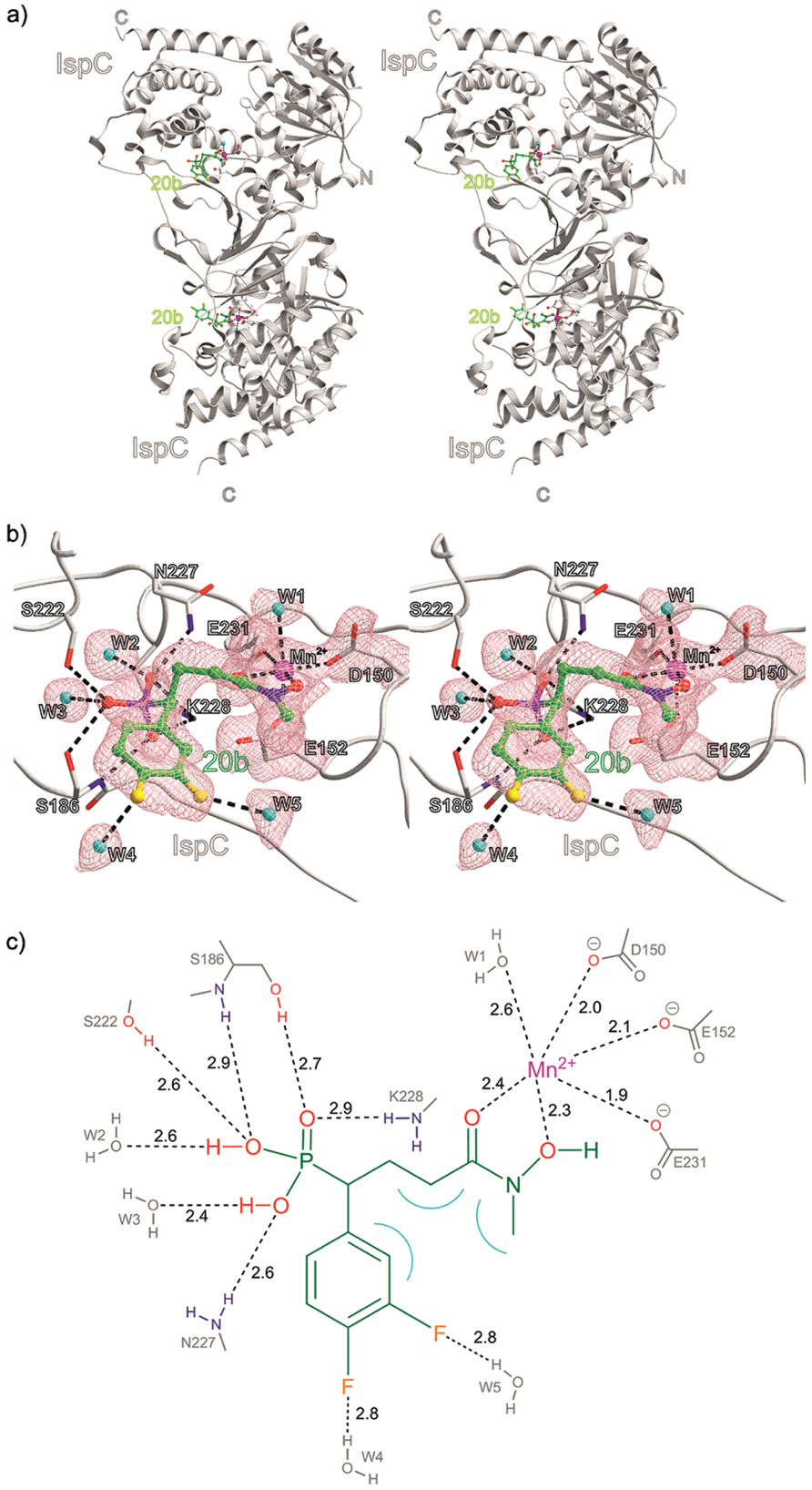

Figure 1. (a) Stereoview of the *Ec*IspC with bound **20b** (PDB code 3R0I). (b) Close-up view of the active site with bound **20b**. The hydrogen-bonding networks at the *Ec*IspC active site including protein, ligand, and water molecules are indicated by dashed black lines. Electron densities represented in purple are contoured at 0.9 Å with $2F_O - F_C$ coefficients. (c) Schematic overview of the interactions (distances are in Å) of **20b** (green) bound to the active site of *Ec*IspC as well as intramolecular van der Waals interactions (light blue) of the *N*-methyl group with the difluorophenyl ring and the main chain atoms of **20b**.

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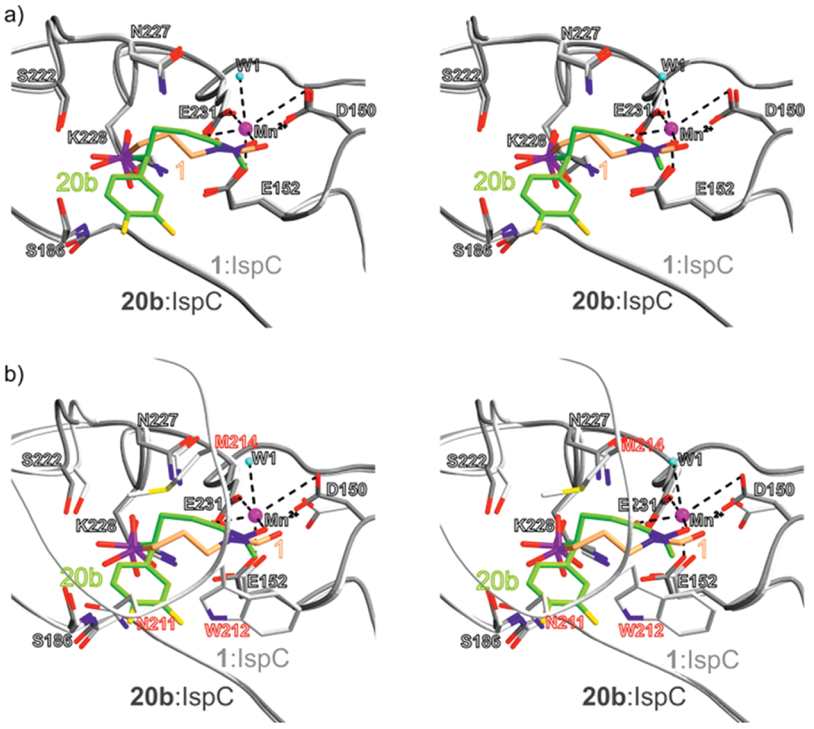

Figure 2. (a) Structural superposition of **20b** and **1** at the active site of *Ec*IspC in the open conformation (PDB codes 3R0I (**20b**) and 1ONP[16]). (b) Superposition of **20b** and **1**/*Ec*IspC/NADPH (closed conformation) complex (PDB codes 3R0I and 1Q0L[17]).

promising candidates for further investigation. In contrast, the bulky *N*-isopropyl substituent of compound **17** caused a complete loss of in vitro activity.

Referring to the selection of aryl substituents in the α-position of the connecting spacer, it is an interesting outcome that the α-naphthyl derivative **20a** inhibits *Pf*IspC in the low nanomolar range with an IC_{50} value of 9.4 nM.

Structure elucidation of *Ec*IspC in complex with **20b** at 2.1 Å showed that the space group of the protein can vary according to the crystallization conditions (Figures 1 and 2).[16−18] The overall structure is virtually identical with other published *Ec*IspC structures except for a flexible loop region (amino acids Arg208−Ser213), which is distorted most likely because of the lack of NADPH. In its closed conformation, this loop stabilizes the substrate or fosmidomycin by hydrophobic interactions.

A structural overlay of the **1**/*Ec*IspC complex with **20b**/*Ec*IspC complex (Figure 2a) documents the consistency of the ligand binding mode.[16] It reveals that, irrespective of the hydroxamic acid in **1** or the reverse hydroxamic acid in **20b**, the coordination of the divalent metal ion is octahedral in both cases. This is reminiscent of the catalytic mechanism of the isomerization of **2**, in which an alcohol and a hydroxyketone are converted into a hydroxyaldehyde (a "reverse hydroxycarbonyl analogue").

Structural superpositioning of the **20b**/*Ec*IspC complex with the **1**/*Ec*IspC/NADPH (closed conformation) complex[17] clearly shows that some amino acid side chains (Figure 2b; only Asn211, Trp212, and Met214 are shown) of the loop region would clash with the difluorophenyl ring of **20b**. This explains why the loop cannot adopt the closed conformation in the **20b** complex where binding of the inhibitor necessitates a major reorientation of the amino acid side chains of the protein. In contrast, binding of **1** occurs in the closed conformation because it perfectly fits into the closed active site. Interestingly, the binding of **20b** in the open conformation might not have suggested that **20b** is, in fact, a stronger inhibitor of the enzyme than **1**, which can bind to the closed conformation.

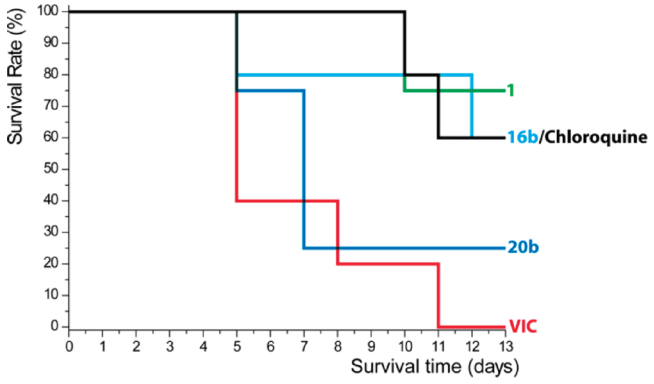

Figure 3. Kaplan−Meier analysis of experiments with mice that had been artificially infected with *P. berghei*: red, control; black, chloroquine (10 mg kg^{-1}); green, **1** (80 mg kg^{-1}); light blue, **16b** (80 mg kg^{-1}); dark blue, **20b** (80 mg kg^{-1}).

The **20b**/*Ec*IspC complex structure reveals intramolecular van der Waals interactions of the *N*-methyl group of **20b** with the difluorophenyl ring (3.9 Å) as well as with the main chain atoms of the ligand. The *N*-methyl group though has no contact to the enzyme.

Thus, **20b** is enthalpically and entropically stabilized despite the missing stabilization through the loop, and ligand binding may thereby be favored. This observation is in good agreement with the obtained in vitro data for compounds **16b** and **20b**. Although the residues in the active site of IspC are conserved between *E. coli* and *P. falciparum*, the overall sequence homology

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Table 2. In Vivo Activity in the *P. berghei* Acute Mouse Model[a]

treatment[b]	mean % suppression of infected RBC (day 4)
vehicle	0
chloroquine (10 mg/kg)[c]	98
1 (80 mg/kg)[c]	92
16b (80 mg/kg)[c]	89
20b (80 mg/kg)[c]	78

[a] GFP ANKA strain. [b] ip for 5 consecutive days. [c] Dose was divided into two administrations of 40 mg kg^{-1} day^{-1}. Ritonavir was coadministered orally at 10 mg kg^{-1} (CYP3A4 inhibitor to minimize possible metabolic degradation.)

is very low. The considerable degree of species specificity that is revealed by our kinetic data emphasizes the necessity to actually use IspC from *P. falciparum* in screening programs for antimalarials directed at IspC.

Compounds **16b** and **20b** were tested in the *Plasmodium berghei* mouse model. Drug treatment of animals was initiated 2 h after artificial infection by intraperitoneal injection of infected erythrocytes and was continued for 5 days. The in vivo efficacy of **16b** was similar to that of **1** and chloroquine (Figure 3). On the other hand, **20b** had less effect on survival, although the percentage of infected erythrocytes was significantly reduced (Figure 3, Table 2).

Thus, whereas the novel compounds show high potency in the nanomolar range when assayed against *P. falciparum* in human erythrocytes and against IspC protein from that pathogen, the small-scale animal study using a different *Plasmodium* species gave less impressive results. It should be noted, however, that in vitro studies on IspC protein of *P. berghei* are not available, and species differences may well be relevant for the different outcomes of the in vitro assays and the animal experiments.

CONCLUSION

In summary, we provide kinetic and crystallographic evidence for the mode of action of a series of novel hydroxamate-based IspC inhibitors. Several new analogues inhibit IspC protein of *P. falciparum* with IC_{50} values in the low nanomolar range. In contrast, the new compounds are significantly weaker inhibitors of the *E. coli* enzyme. Finally, pilot in vivo experiments have shown that compound **16b** has some potential and is believed to be a promising candidate for further pharmacological studies, structural modifications, and the design of prodrugs.

EXPERIMENTAL SECTION

General Procedures. All solvents and chemicals were used as purchased without further purification. The progress of all reactions was monitored on Merck precoated silica gel plates (with fluorescence indicator UV_{254}) using ethyl acetate/*n*-hexane as solvent system. Column chromatography was performed with Fluka silica gel 60 (230−400 mesh ASTM) with the solvent mixtures specified in the corresponding experiment. Spots were visualized by irradiation with ultraviolet light (254 nm). Melting points (mp) were taken in open capillaries on a Mettler FP 5 melting-point apparatus and are uncorrected. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance 500 (500.13 MHz for ^{1}H; 125.76 MHz for ^{13}C) using DMSO-d_6 and $CDCl_3$ as solvents. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H and ^{13}C and to external tetramethylsilane). Elemental analysis was performed on a Perkin-Elmer PE 2400 CHN elemental analyzer. IR spectra were recorded on a Varian 800 FT-IR Scimitar series. Analytical high pressure liquid chromatography (HPLC) was performed in analogy to a previously reported procedure.[11k] The instrument was an Elite LaChrom system [Hitachi L-2130 (pump) and L-2400 (UV-detector)]. The column was a Phenomenex Luna C-18(2), 1.8 μm particle (250 mm × 4.6 mm), supported by Phenomenex Security Guard Cartridge Kit C18 (4.0 mm × 3.0 mm). The purity of all final compounds was 95% or higher.

Experimental Data for Compounds. Experimental data are listed below for selected compounds **10b**, **19b**, and **20b**.

General Procedure for the Synthesis of O-Bn-Protected Hydroxamic Acids (9a,b, 10a−c, 11, 12). To a solution of the respective carboxylic acid **8a−d** (1 equiv, 5 mmol) in dry CH_2Cl_2 (50 mL) was added 1,1′-carbonyldiimidazole (1.1 equiv, 0.9 g, 5.5 mmol) in small portions. After the mixture was stirred at room temperature for 45 min, the appropriate hydroxylamine was added in one portion. The solution was stirred overnight, and the solvent was removed under reduced pressure. The remaining residue was dissolved in ethyl acetate (30 mL), and the organic layer was washed three times with an aqueous solution of citric acid (10%, 10 mL) and once with a saturated aqueous solution of $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Hydroxamic acids (**9a**, **10b**, and **10c**) were obtained by crystallization from diethyl ether at 7 °C. Hydroxamic acids **9b**, **10a**, and **11** were purified by column chromatography on silica gel using ethyl acetate/*n*-hexane (1:1) as the eluent.

Diethyl {4-[(Benzyloxy)(methyl)amino]-1-(3,4-difluorophenyl)-4-oxobutyl}phosphonate (10b). White solid (2.10 g, 92%). Mp: 60.2 °C. ^{1}H NMR (500.13 MHz, DMSO-d_6): δ = 7.61−7.28 (m, 5H), 7.28−7.01 (m, 3H), 4.71 ($PhCH_2$, s, 2H), 4.25−3.92 (CH_3CH_2, m, 2H), 3.92−3.70 (CH_3CH_2, m, 2H), 3.34−3.22 (PCH, dd, J_1 = 11.0 Hz, J_2 = 24.0 Hz, 1H), 3.10 (NCH_3, s, 3H), 2.42−2.07 (CH_2, m, 3H), 2.08−1.76 ($CHCH_2$, m, 1H), 1.20 (CH_3, t, *J*=6.9 Hz, 3H), 1.06 (CH_3, t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 172.65 (C═O), 149.07 (dd, $^2J_{C-F}$ = 14.1 Hz, $^1J_{C-F}$ = 246.9 Hz), 148.43 (dd, $^2J_{C-F}$ = 12.0 Hz, $^1J_{C-F}$ = 242.7 Hz), 134.38, 133.97, 129.27, 128.58, 128.24, 125.92, 117.87 (dd, $^3J_{C-F}$ = 6.5 Hz, $^2J_{C-F}$ = 17.4 Hz), 117.26 (dd, $^3J_{C-F}$ = 1.4 Hz, $^2J_{C-F}$ = 16.7 Hz), 75.08 ($PhCH_2$), 61.73 ($POCH_2$, d, $^2J_{C-P}$ = 6.8 Hz), 61.45 ($POCH_2$, d, $^2J_{C-P}$ = 7.1 Hz), 41.28 (CHP, d, $^1J_{C-P}$ = 136.9 Hz), 32.63 (NCH_3), 29.17 (CH_2, d, $^2J_{C-P}$ = 15.5 Hz), 24.31 (CH_2), 16.14 (CH_3, d, $^3J_{C-P}$ = 5.5 Hz), 15.99 (CH_3, d, $^3J_{C-P}$ = 5.4 Hz) ppm. IR (KBr): $\tilde{\nu}$ = 3037 (C−$H_{arom.}$), 2985 (C−$H_{aliph.}$), 1673 (C═O), 1233 (P═O), 1053 (P−O) cm^{-1}. Anal. Calcd for $C_{22}H_{28}F_2NO_5P$: C 58.02, H 6.20, N 3.08. Found: C 58.29, H 6.29, N 2.78.

General Procedure for the Synthesis of Phosphonic Acids (19a−c). To a solution of the respective phosphonic acid diethyl ester **10a−c** (1 equiv, 3 mmol) in dry dichloromethane (10 mL) was added trimethylsilyl bromide (5 equiv, 1.99 mL, 15 mmol) at 0 °C. After 1 h, the solution was allowed to warm to room temperature and stirred for an additional 23 h. The solvent was removed under reduced pressure. The remaining residue was dissolved in THF (10 mL), and water (0.1 mL) was added. After 30 min the solvent was evaporated and the resulting residue dried in vacuo overnight. Pure phosphonic acids (**19a−c**) were obtained as white solids at −20 °C after digestion with ethyl acetate.

{4-[(Benzyloxy)(methyl)amino]-1-(3,4-difluorophenyl)-4-oxobutyl}phosphonic Acid (19b). White solid (0.480 g, 40%). Mp: 143.5 °C. ^{1}H NMR (500.13 MHz, DMSO-d_6): δ = 7.62−7.15 (m, 7H), 7.08 (s, 1H), 4.70 ($PhCH_2$, s, 2H), 3.09 (NCH_3, s, 3H), 2.94 (PCH, dd, J_1 = 11.0 Hz, J_2 = 20.2 Hz, 1H), 2.22 (CH_2, s, 3H), 1.94 (CH_2, m, 1H) ppm. ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 172.66 (C═O), 149.02 (dd, $^2J_{C-F}$ = 12.7 Hz, $^1J_{C-F}$ = 233.9 Hz), 148.12 ($^2J_{C-F}$ = 13.3 Hz, $^1J_{C-F}$ = 228.1 Hz), 136.11 (d, $^2J_{C-P}$ = 8.8 Hz), 134.39, 129.29, 128.58, 128.23, 125.80, 117.57 (dd, $^3J_{C-F}$ = 5.7 Hz, $^2J_{C-F}$ = 16.5 Hz), 116.84 (dd, $^2J_{C-F}$ = 16.4 Hz), 75.06

($PhCH_2$), 43.53 (CHP, d, $^1J_{C-P}$ = 133.4 Hz), 32.68 (NCH_3), 29.57 (CH_2, d, $^2J_{C-P}$ = 16.8 Hz), 24.90 (CH_2, d, $^3J_{C-P}$ = 2.1 Hz) ppm. IR (KBr): $\tilde{\nu}$ = 3437 (N−H), 3037 (C−$H_{arom.}$), 2944 (C−$H_{aliph.}$), 1607 (C═O), 1277 (P═O), 999 (P−O) cm^{-1}. Anal. Calcd for $C_{18}H_{20}F_2NO_5P$: C 54.14, H 5.05, N 3.51. Found: C 54.02, H 5.06, N 3.23.

General Procedure for the Synthesis of Target Compounds (16a,b, 17, 18, and 20a−c). To a solution of the appropriate *O*-Bn-protected acid (1 mmol) in freshly distilled methanol (20 mL) was added Pd−C catalyst (10%, 40 mg). The mixture was hydrogenated at a pressure of 2 bar for 1.5 h (in the case of compounds **13a,b**, **14**, and **15**) or 3 h (in the case of compounds **19a−c**). The suspension was filtered through an SPE tube RP-18, and the solvent was removed under reduced pressure. Whereas compounds **17**, **18**, and **20c** were obtained as hygroscopic oils, compounds **16a,b** and **20a,b** were crystallized from appropriate solvents as described below.

{1-(3,4-Difluorophenyl)-4-[hydroxy(methyl)amino]-4-oxobutyl}phosphonic Acid (20b). White solid (0.258 g, 84% after recrystallization in ethyl acetate). Mp: 116.8 °C. ^{1}H NMR (500.13 MHz, DMSO-d_6): δ = 9.67 (NOH, s, 1H), 7.35 (dd, J_1 = 8.9 Hz, J_2 = 19.0 Hz, 1H), 7.30−7.22 (m, 1H), 7.18−6.86 (m, 1H), 3.02 (NCH_3, s, 3H), 2.97 (PCH, dd, J_1 = 9.6 Hz, J_2 = 21.7 Hz, 1H), 2.20 (CH_2, s, 3H), 1.98−1.82 ($CHCH_2$, m, 1H) ppm. ^{13}C NMR (125.76 MHz, DMSO-d_6): δ=172.20 (C═O), 149.76 (dd, $^2J_{C-F}$ = 10.8 Hz, $^1J_{C-F}$ = 242.9 Hz), 148.96 (dd, $^2J_{C-F}$ = 13.9 Hz, $^1J_{C-F}$ = 250.6 Hz), 136.13, 125.82, 117.54 (dd, $^3J_{C-F}$ = 5.8 Hz, $^2J_{C-F}$=16.9 Hz), 116.77 (dd, $^3J_{C-F}$ = 1.5 Hz, $^2J_{C-F}$ = 16.6 Hz), 43.61 (CHP, d, $^1J_{C-P}$ = 133.4 Hz), 35.54 (NCH_3), 29.69 (CH_2, d, $^2J_{C-P}$ = 15.1 Hz), 25.00 (CH_2) ppm. IR (KBr): $\tilde{\nu}$ = 3405 (N−H), 1615 (C═O), 1282 (P═O), 1019 (P−O) cm^{-1}. Anal. Calcd for $C_{11}H_{14}F_2NO_5P$: C 42.73, H 4.56, N 4.53. Found: C 43.00, H 4.77, N 4.50.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, analytical data, enzyme assays, biological evaluation of in vitro antiplasmodial activity and cytotoxicity, in vivo mouse model with *P. berghei*, and experimental data regarding crystallization and structure determination. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

‡PDB code for *Ec*IspC with bound **20b** is 3R0I.

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Author Contributions

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■ DEDICATION

†Dedicated to Dr. Viktoriya Illarionova, in memoriam.

■ ABBREVIATIONS USED

n-BuLi, *n*-butyllithium; BW, body weight; DCC, dicyclohexylcarbodiimide; DMF, *N*,*N*-dimethylformamide; DOXP, 1-deoxy-D-xylulose 5-phosphate; Dxr (IspC), 1-deoxy-D-xylulose 5-phosphate reductoisomerase; ip, intraperitoneal; MEP, 2*C*-methyl-D-erythritol 4-phosphate; MRC-5, human fetal lung fibroblast; NADPH, nicotinamide adenine dinucleotide phosphate; Pd−C, palladium on activated carbon; RBC, red blood cell; RT, room temperature; SD, standard deviation; THF, tetrahydrofuran; TMSBr, bromotrimethylsilane

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