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α-Substituted β-Oxa Isosteres of Fosmidomycin: Synthesis and Biological Evaluation

Karin Brücher,[†] Boris Illarionov,[§] Jana Held,[∥] Serena Tschan,[∥] Andrea Kunfermann,[‡] Miriam K. Pein,[†] Adelbert Bacher,[‡] Tobias Gräwert,[§] Louis Maes,[⊥] Benjamin Mordmüller,[∥,▽] Markus Fischer,[§] and Thomas Kurz*,[†]

[†]Institut für Pharmazeutische und Medizinische Chemie, Heinrich Heine Universität, Universitätsstr. 1, 40225 Düsseldorf, Germany

[§]Hamburg School of Food Science, Universität Hamburg, Grindelallee 117, 20146 Hamburg, Germany

[∥]Institut für Tropenmedizin, Eberhard Karls Universität Tübingen, Wilhelmstr. 27, 72074 Tübingen, Germany

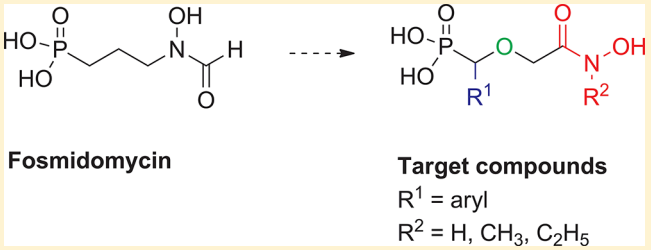
[‡]Center for Integrated Protein Science Munich, Lehrstuhl für Biochemie, Technische Universität München, Lichtenbergstr. 4, 85747 Garching, Germany

[⊥]Laboratory for Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Groenenborgerlaan 171, 2020 Wilrijk, Belgium

[▽]Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon

Supporting Information

ABSTRACT: Specific inhibition of enzymes of the non-mevalonate pathway is a promising strategy for the development of novel antiplasmodial drugs. α-Aryl-substituted β-oxa isosteres of fosmidomycin with a reverse orientation of the hydroxamic acid group were synthesized and evaluated for their inhibitory activity against recombinant 1-deoxy-D-xylulose 5-phosphate reductoisomerase (IspC) of *Plasmodium falciparum* and for their *in vitro* antiplasmodial activity against chloroquine-sensitive and resistant strains of *P. falciparum*. The most active derivative inhibits IspC protein of *P. falciparum* (*Pf*IspC) with an IC_{50} value of 12 nM and shows potent *in vitro* antiplasmodial activity. In addition, lipophilic ester prodrugs demonstrated improved *P. falciparum* growth inhibition *in vitro*.



INTRODUCTION

The spread of *Plasmodium falciparum* resistance to most antimalaria drugs is a major challenge in the control of malaria.[1] Today, resistance affects nearly all main classes of antimalarial drugs, and until now, artemisinin derivatives and artemisinin combination therapy remain the workhorse for the treatment of severe and uncomplicated malaria, respectively. Currently, the situation is alarming, because decelerated parasite elimination after artemisinin therapy is reported from Southeast Asia, a possible indicator for the development of resistance.[2] Despite the urgent need for new antimalarials, only a very few non-artemisinin drug candidates are currently in clinical development. Most of these drug candidates are variations of existing antimalarial drugs.[3] Hence, the development of new classes that exhibit novel modes of action is of utmost importance. Currently, only very few drug candidates meet these criteria. Among them and of particular interest is the phosphonohydroxamic acid antibiotic fosmidomycin (**1**), which was isolated from *Streptomyces lavendulae* in the late 1970s.[4] Due to its unique mechanism of action, cross-resistance between **1** and other commonly used antimalarials is unlikely. Clinical studies conducted with **1** in combination with clindamycin, a slow-acting partner drug, have already demonstrated good efficacy in the treatment of acute uncomplicated *P. falciparum* malaria.[5] However, due to its high polarity and its deprotonation at physiological pH values, **1** has low oral bioavailability of approximately 10−30% and a short plasma half-life of approximately 1.9 h.[6]

1 exerts its antiplasmodial activity by inhibition of 2*C*-methyl-D-erythritol synthase (IspC/Dxr), which catalyzes the first committed step of the non-mevalonate isoprenoid biosynthesis pathway.[7] Since that pathway is absent in mammalians, which produce isoprenoid precursors via the mevalonate pathway, target-based adverse effects are not to be expected.[8] To improve the properties of **1**, bioisosteric replacements of both functional groups, modification of the aliphatic spacer as well as the design of phosphonate prodrugs have been reported.[9] Recently, some analogues with a reverse orientation of the hydroxamic acid functionality have been described (**3a**−**d**), which inhibit IspC protein of *P. falciparum* (*Pf*IspC) with IC_{50} values in the low nanomolar range (Scheme 1).[10] In a previous publication, we provided kinetic and crystallographic evidence for their mode of action. Pilot *in vivo*

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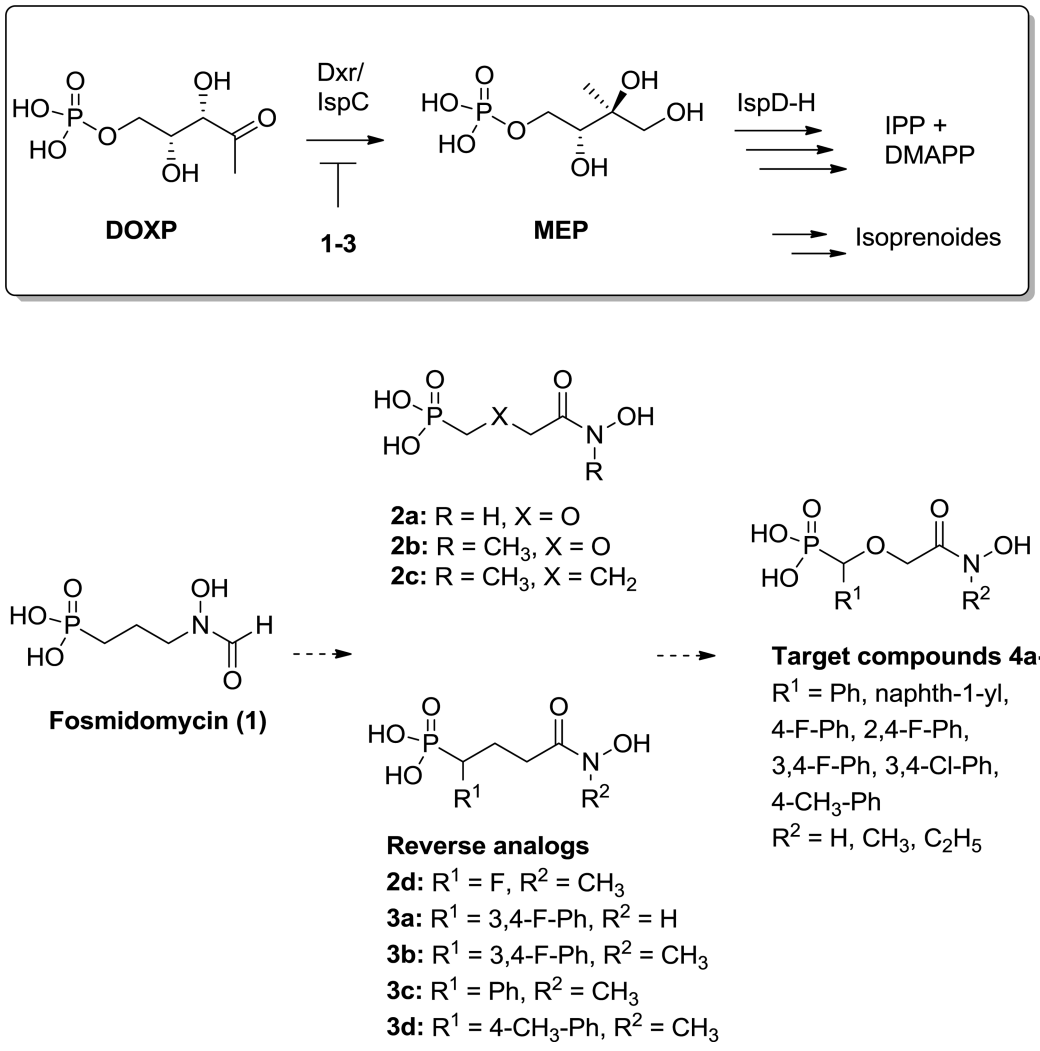
Scheme 1. Methylerythritol Phosphate (MEP) Pathway and Chain-Modified Target Compounds



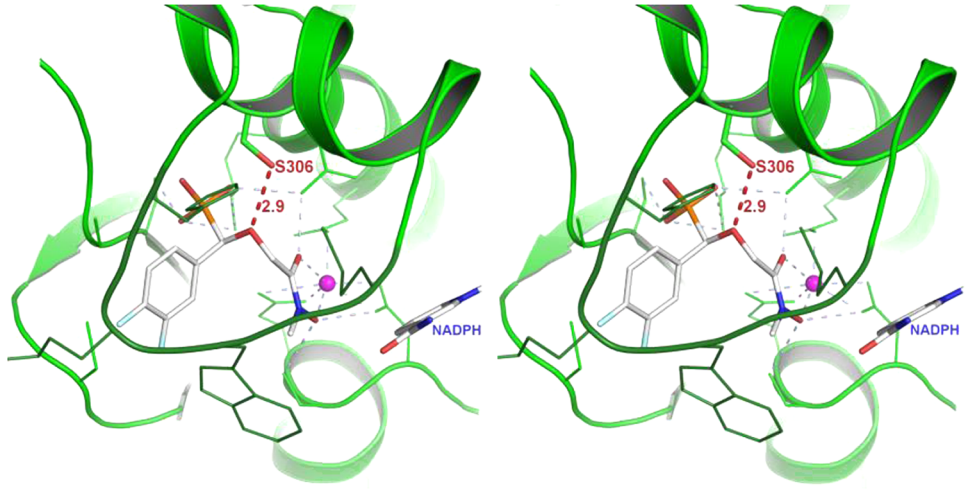


Figure 1. Stereo representation of a hypothetical model of compound **4e** into the active site of *Pf*IspC. **4e** (white), NADPH (white), and S306 (green) are shown in stick representation. Additional amino acid residues at the active site are shown in line representation. A hypothetical H-bond (2.9 Å) between Oγ of S306 and the β-oxa group of **4e** is shown as dashed red line. Atomic coordinates for the carba analogue of **4e** were taken from PDB file 3R0I, and the β-methylene group was replaced with oxygen. The coordinates of **4e** were then merged with 3AU9, replacing the coordinates of **1**. The ensemble was minimized with *SYBYLX 2.0*.

experiments have also demonstrated *in vivo* potential for compound **3a**.[10b]

In an attempt to find derivatives of **1** with further improved activity, we synthesized a small library of reverse β-oxa isosteres

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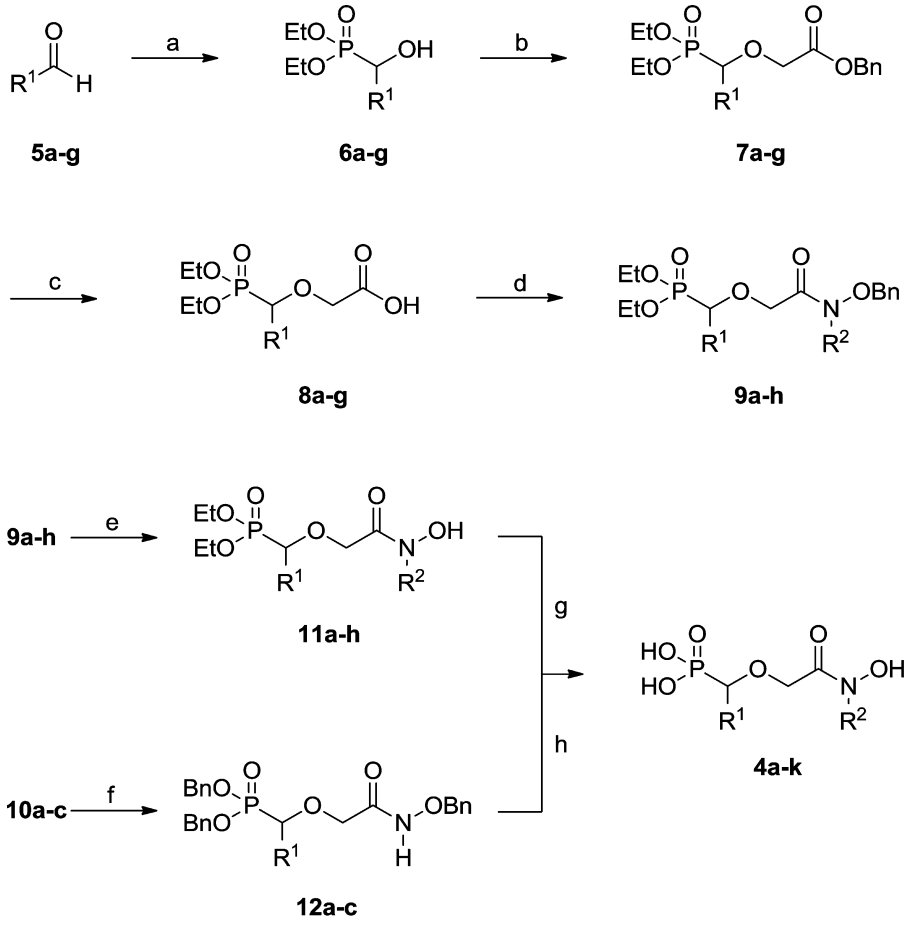
Scheme 2. Synthesis of Target Compounds 4[a]



4a-g,9a-g,11a-g: $R^2 = CH_3$, R^1 = Ph, naphth-1-yl, 4-F-Ph, 2,4-F-Ph, 3,4-F-Ph, 3,4-Cl-Ph, 4-CH_3-Ph
4h, 9h,11h: $R^2 = C_2H_5$, R^1 = Ph
4i-k,10a-c,12a-c: R^2 = H, R^1 = Ph, 2,4-F-Ph, 4-CH_3-Ph
5a-g, 6a-g, 7a-g, 8a-g: R^1 = Ph, naphth-1-yl, 4-F-Ph, 2,4-F-Ph, 3,4-F-Ph, 3,4-Cl-Ph, 4-CH_3-Ph

[a]Reagents and conditions: (a) DEP, TEA, RT, 12 h, 74−99%; (b) $BrCH_2CO_2Bn$, Ag_2O, DMF, RT, 24 h, 40−83%; (c) H_2, Pd−C, MeOH or ethyl acetate, 1 atm, RT, 2 h, 75−99%; (d) CDI, $BnONHR^2$ (R^2 = H, CH_3, C_2H_5), CH_2Cl_2, RT, 12 h, 85−98%; (e) H_2, Pd−C, MeOH or ethyl acetate, 1 atm, RT, 2 h, 73−99%; (f) 1. TMSBr, CH_2Cl_2, RT, 24 h; 2. THF/H_2O, RT, 1 h; 3. DCC, BnOH, benzene, 80 °C, 4 h, 25−26% (3 steps); (g) 1. TMSBr, CH_2Cl_2, RT, 24 h; 2. THF/H_2O, RT, 1 h, 21−85% (2 steps); (h) H_2, Pd−C, MeOH, 1 atm, RT, 2 h, 99%.

(**4a**−**k**) and determined their inhibitory activity in the *Pf*IspC enzyme assay and in continuous *P. falciparum in vitro* culture.

RESULTS

Earlier studies had shown that the introduction of differently substituted phenyl residues at the α-position of **1** can significantly increase the inhibitory potency toward *Pf*IspC.[10a,b] It has also been shown that the replacement of the β-methylene group of **1** by oxygen resulted in improved efficacy against *P. falciparum* laboratory isolates.[9j] It was therefore appropriate to investigate whether the combination of these respective structural modifications (i.e., aromatic substituents at the α-position of β-oxa isosteric compounds) results in improved activity at the level of the isolated enzyme target and at the level of a whole cell *in vitro* assay.

In order to analyze whether the replacement of the β-methylene group of the previously reported compound **3b**[10b] by oxygen might enable the formation of an additional hydrogen bond with *Pf*IspC and a consecutive increase in affinity, we performed a modeling study using the X-ray structure of *Pf*IspC in complex with **1**, NADPH and a metal ion as template (RCSB accession code, 3AU9; the use of the quaternary complex including inhibitor, coenzyme, and metal ion appeared crucial for a comparison between kinetic and structural data, since kinetic studies are by necessity performed in the presence of coenzyme and metal ions). **1** in 3AU9 was replaced by compound **3b** in the conformation that it assumes in the complex with IspC of *Escherichia coli* (*Ec*IspC; PDB accession code, 3R0I),[10b] and the β-methylene group of the ligand was replaced by oxygen (affording the hypothetical ligand structure **4e**). Minimization of the hybrid model supported the potential formation of a hydrogen bond between the oxygen in the β-position of the hypothetical **4e** ligand and the highly conserved Ser306 (*P. falciparum* numbering) side chain (Figure 1). On this basis, we decided to synthesize a small

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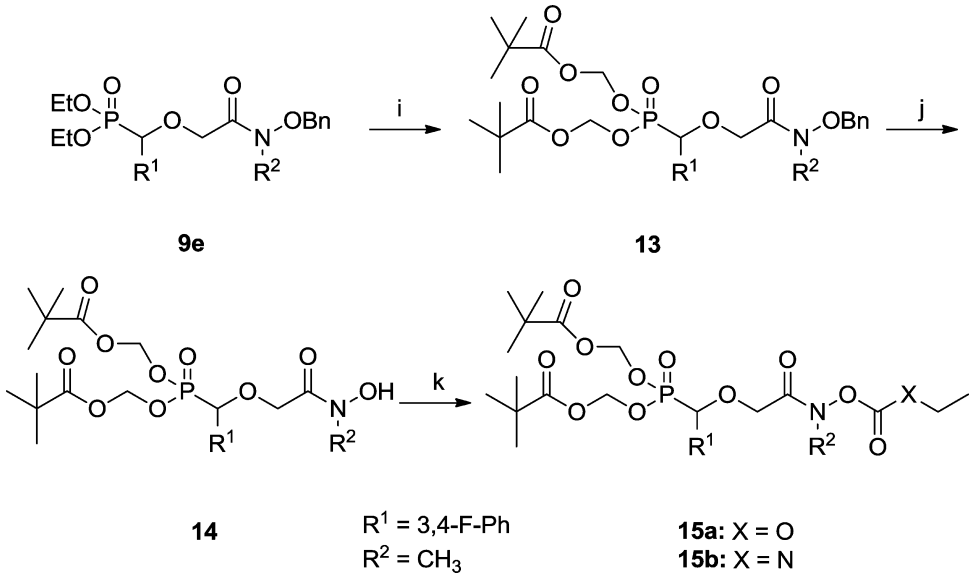
Scheme 3. Synthesis of Prodrugs 14 and 15[a]



[a]Reagents and conditions: (i) 1. TMSBr, CH_2Cl_2, RT, 24 h; 2. THF/H_2O, RT, 1 h; 3. chloromethyl pivalate, TEA, DMF, 80 °C, 6 h, 37% (3 steps); (j) H_2, Pd–C, MeOH, 1 atm, RT, 2 h, 99%; (k) ethyl chloroformate or ethyl isocyanate, TEA, CH_2Cl_2, RT, 1 h, 93–98%.

library of β-oxa isosteres (**4**) of previously published derivatives of **1** with known inhibitory activity (**3**).[10]

Synthesis of β-Oxa Isosteres. A retrosynthetic analysis led to α-hydroxyphosphonates (**6**) and commercially available benzyl bromoacetate as suitable building blocks for the synthesis of target compounds **4a–k**. Previously reported and unreported α-hydroxyphosphonates **6a–g** were accessible by Pudovik reactions of aromatic aldehydes **5a–g** with diethyl phosphite in the presence of triethylamine according to established literature procedures.[11] *O*-Alkylation of **6a–g** with benzyl bromoacetate and neutral silver oxide led to completely protected synthones 7**a–j** in good yields.[12] Next, benzyl esters **7a–g** were deprotected by catalytic hydrogenation to afford carboxylic acids **8a–g**. 1,1′-Carbonyldiimidazole (CDI)-mediated activation of **8a–g** followed by treatment with various *O*-benzyl-protected hydroxylamines provided the protected phosphonohydroxamic acids **9a–h** and **10a–c**.[13] The corresponding free hydroxamic acids **11a–h** were obtained in good yields and purity by catalytic hydrogenation of **9a–h**.

Cleavage of the phosphonic ester group of compounds **11** was accomplished by dealkylation with bromotrimethylsilane and subsequent aqueous hydrolysis.[14] In the case of the *N*-alkyl-substituted derivatives **11a–h**, the desired target compounds **4a–h** were obtained as pure solids, whereas compounds **4i–k** were isolated as oily and hygroscopic mixtures of **4** and **11**. Therefore, the phosphonic acid diethyl ester moieties of substrates **10a–c** were converted into the corresponding dibenzyl phosphonate moieties using a transesterification method.[15] Finally, catalytic hydrogenation of Bn-protected precursors **12a–c** afforded free hydroxamic acids **4i–k**.

Synthesis of Prodrugs of 4e. Deprotection of the phosphonic acid moiety of intermediate **9e** was accomplished by dealkylation and subsequent hydrolysis as described before. Without prior purification, the crude phosphonic acid was alkylated by reaction with chloromethyl pivalate in the presence of triethylamine according to published procedures.[9i,j]

Next, catalytic hydrogenation of benzyl ester **13** led to prodrug **14**. Finally, the hydroxamic acid moiety of **14** was reacted with ethyl chloroformate or ethyl isocyanate in the presence of triethylamine to yield a new type of lipophilic ester prodrug **15a,b**.[16]

Biological Studies. The β-oxa isoster minilibrary was assayed with recombinant *Pf*IspC using published procedures.[10a,b] The experimental data can be described by a competitive kinetic model without cooperative interactions (Figure 2).[17] IC_{50} values are summarized in Table 1 and show a

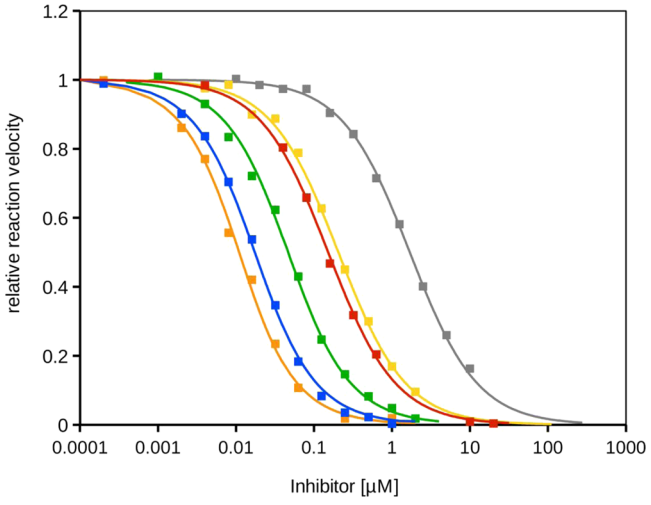


Figure 2. Inhibition of *Pf*IspC; **1** (red), **4b** (green), **4c** (blue), **4f** (orange), **4h** (yellow), and **4k** (gray).

range of 0.012 μM to >50 μM (Figure 2). The table also shows IC_{50} values obtained by growth assay with *P. falciparum* using the chloroquine-sensitive strain 3D7 and the multiresistant strain Dd2.

IC_{50} values against the chloroquine-sensitive strain 3D7 and the chloroquine-resistant strain Dd2 are visualized in Figure 3A.

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Table 1. Inhibition of *Pf*IspC and *in Vitro* Antiplasmodial Activity

compound	R^1	R^2	X	*Pf*IspC[a,c] IC_{50} [μM]	*Pf*Dd2[b,c] IC_{50} [μM]	*Pf*3D7[b,c] IC_{50} [μM]
1				0.16 ± 0.02	0.81	0.88
4a	Ph	CH_3		0.037 ± 0.002	0.70	1.2
4b	naphth-1-yl	CH_3		0.039 ± 0.004	0.35	0.52
4c	4-F-Ph	CH_3		0.027 ± 0.001	0.35	0.48
4d	2,4-F-Ph	CH_3		0.065 ± 0.005	1.2	1.3
4e	3,4-F-Ph	CH_3		0.012 ± 0.001	0.13	0.54
4f	3,4-Cl-Ph	CH_3		0.014 ± 0.001	0.14	0.24
4g	4-CH_3−Ph	CH_3		0.025 ± 0.003	0.19	0.18
4h	Ph	C_2H_5		0.21 ± 0.01	3.8	8.1
4i	Ph	H		1.5 ± 0.1	n. d.	>50
4j	2,4-F-Ph	H		1.7 ± 0.1	n. d.	>50
4k	4-CH_3−Ph	H		1.8 ± 0.1	n. d.	>50
14	3,4-F-Ph	CH_3		n.d.	0.022	0.013
15a	3,4-F-Ph	CH_3	O	n.d.	0.019	0.023
15b	3,4-F-Ph	CH_3	N	n.d.	0.022	0.031
3b	3,4-F-Ph	CH_3		0.0034 ± 0.0004	n. d.	n. d.
3c	Ph	CH_3		0.0031 ± 0.0003	n. d.	n. d.
3d	4-CH_3−Ph	CH_3		0.014 ± 0.002	n. d.	n. d.

[a]Values (mean ± SD calculated from nine or more data points) were determined using nonlinear regression analysis as described earlier.[18] [b]Values are the mean of at least two independent experiments conducted in duplicate, each using 12 serial dilutions. [c]n.d., not determined; antiplasmodial *in vitro* activity of **3b−d** toward *Pf*K1 strain was published previously.[10]

The differences between the results obtained with the respective strains are quite small; in the double logarithmic plot, all values lie close to the line of best fit (dashed red line in Figure 3A).

The IC_{50} values for the enzyme assay and the *in vitro* growth assay with *P. falciparum* are correlated graphically in Figure 3B. Notably, for the β-oxa analogues of **1**, the IC_{50} values of the enzyme assay are lower than those from the growth inhibition assay; the values show an excellent correlation coefficient of 0.98 and can be roughly correlated by a parallel to the image diagonal.

In order to unequivocally assess the impact of the β-oxa replacement, it is of course crucial to compare the data in Table 1 with those for the cognate derivatives of **1** with methylene groups in the β position (subsequently designated as "carba" analogues, **3a−d**). The data for the "carba series" (from our previous work: Scheme 1, Table 1) were published previously.[10] **3b−d** are also shown graphically in Figure 3C. The abscissa in this graph is the $\log_{10}$ of the IC_{50} value of the β-oxa series. The ordinate is the difference between the logarithm of the IC_{50} values of the cognate oxa:carba pairs. The data leave no doubt that, contrary to our initial hypothesis, the oxa compounds are weaker inhibitors as compared to the cognate carba compounds. The losses in binding free enthalpy caused by the oxygen substitution are in the range of about 1 kcal mol^{-1} for the compounds shown in red and above 1 kcal mol^{-1} for the compounds shown in green in Figure 3C (even though we are using IC_{50} data rather than K_i values, the ordinate values are approximately equivalent to $\Delta\Delta G_{binding}$, i.e., the free enthalpy difference between each respective oxa and carba compound in the bound state). The derivatives differ by the presence (red) or absence (green) of an alkyl substituent (typically methyl). The marked difference between these groups prompted further analysis, which was possible since the minilibrary contained several pairs of compounds which differ only by the presence or absence of the *N*-alkyl substituent. In Figure 3D, the abscissa represents IC_{50} values of compounds from either the carba or oxa series. The ordinate represents the difference of the logarithms of IC_{50} of the *N*-methylated and non-methylated compound, respectively (which is related to $\Delta\Delta G_{binding}$ of methylated and non-methylated partner). The values for cognate pairs in the carba series are shown as triangles, and the values for the oxa pairs are shown as squares. The data show that *N*-methyl substitution causes a gain in binding enthalpy ranging from about 0 to 2 kcal mol^{-1}.

Earlier, we could show that the efficacy of α-aryl substituted analogues of **1** could be improved by conversion into phosphonate ester prodrugs.[9f,h] On the basis of that experience, we prepared several prodrugs of compound **4e** which documented impressively improved efficacy in *P. falciparum* growth assays.

DISCUSSION

1 is exceptional since it is the only new non-artemisinin drug candidate in clinical development that may complement artemisinin combinations in the treatment of uncomplicated malaria. In an attempt to improve the affinity of existing derivatives of **1** for *Pf*IspC, we prepared a series of β-oxa isosteres with the expectation that the formation of an additional hydrogen bond might afford an increase in affinity. However, the comparison of two minilibraries whose cognate members differed only by the presence of oxygen or methylene groups in the β position showed that the β oxygen reduced the binding free enthalpy by about 1 kcal mol^{-1}. Attempts to co-crystallize one of the oxa derivatives with *Pf*IspC were as yet unsuccessful. Thus, it remains unknown whether the expected additional hydrogen bond fails to be formed, or whether it is indeed formed but does not improve the affinity due to some structural penalty, possibly due to interference with the network of other hydrogen bonds at the active site. As a side issue, the data also show clearly that the *N*-methyl derivatives are significantly better inhibitors than the unmethylated analogues.

After conversion to lipophilic ester prodrugs (**14**, **15a,b**), the best oxa isosteres had significantly better IC_{50} values (0.013−

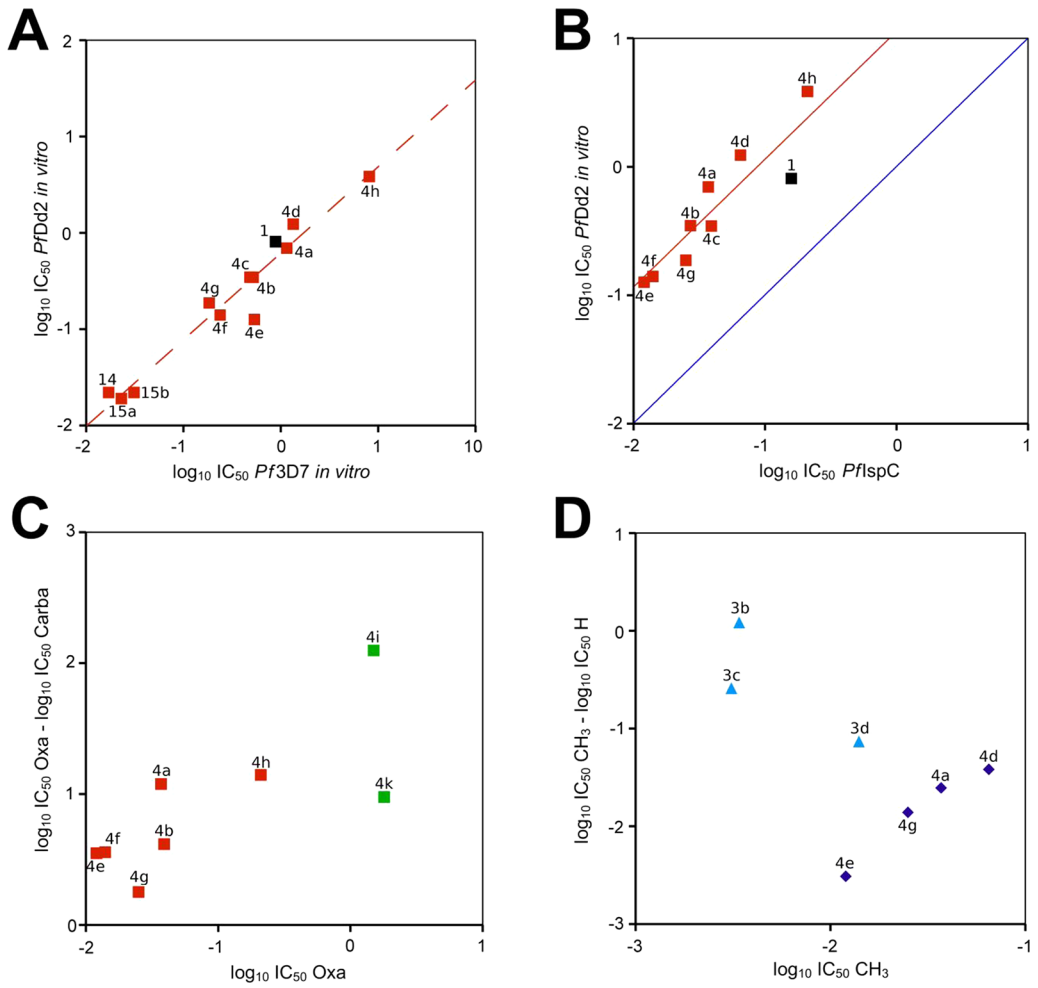

Figure 3. (a) Activities against the chloroquine resistant strain Dd2 versus activities against strain 3D7. The line of best fit is close to the image diagonal; black, fomidomycin. (b) Correlation of enzyme assay and cell-based growth inhibition assay. Most substances lie close to a red line that is shifted from the image diagonal (blue) by 1 order of magnitude in the *y* direction (no line of best fit is shown in this graph); black, **1**. (c,d) All values originate from IspC enzyme assays. (c) Difference between the $\log_{10}$ of the IC_{50} value of a given oxa substance and the $\log_{10}$ of the IC_{50} of the respective carba derivative versus $\log_{10}$ of the IC_{50} of the oxa substance; red, methyl or ethyl group in position R^2; green, hydrogen in position R^2. (d) Difference between the $\log_{10}$ of the IC_{50} value of a given *N*-methyl-substituted compound (R^2) and the $\log_{10}$ of the IC_{50} of the cognate nonalkylated substance (R^2) versus $\log_{10}$ of the IC_{50} of the respective *N*-methylated substance. Light blue: carba analogues; magenta: oxa analogues.

0.031 μM) in the *P. falciparum* growth assay than **1** (Table 1). This is in line with earlier favorable reports on pivaloyloxymethyl ester prodrugs of derivatives of **1**. However, many details of the successful prodrug concept are still incompletely understood. As a surrogate marker for the efficacy of derivatives of **1** as antimalarials, enzyme assays with the molecular target *Pf*IspC have the advantage of high reproducibility and accuracy. When this biochemical precision tool can be applied to families of structurally similar compounds, the impact of minimal structural changes on the binding enthalpy can be assessed in minute detail. Using this approach, we found that, contrary to expectation, the replacement of the methylene group in the β-position of the linker between the phosphonate and hydroxamate motifs by an oxygen atom is accompanied by an affinity penalty in the range of about 1 kcal mol^{-1}. We also found that methylation of the hydroxamic acid pharmacophore is accompanied by a gain in affinity of similar size. Notably, the effect of introducing oxygen in the β-position and of methylation are not correlated in a simple way. The impact of methylation is much larger in the case of the oxa series as compared to the carba series. The combination of methylene in the β-position and methyl at the hydroxamic acid motif gives the highest affinity. Clearly, only the detailed kinetic analysis of multiple analogues has the potential to unravel these details which are "below the observational horizon" of a purely structure-based approach, even in the case of optimal crystallographic resolution.

The antimalarial activity of derivatives of **1** cannot be predicted solely by *Pf*IspC enzyme assays because the situation in the intact organism may have a strong impact on activity and on-target concentration. Since the enzyme is located inside the apicoplast, a plastid that arose through secondary endosymbiosis in apicomplexan protozoa, the inhibitor has to pass the erythrocyte membrane and 5 to 7 lipid membranes of the parasite in order to reach its molecular target.[19] Notably, it is as yet unknown how and where the prodrugs are hydrolytically converted to the free phosphonic acids.

Moreover, recent work suggests that the membrane of the erythrocyte host cell allows the passage of (genuine, unmodified) **1** only after it has been modulated by the presence of the intracellular pathogen.[20] Enteral resorption and bioavailability are further factors that must be taken into

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account. Even though enzyme inhibition at single-digit nanomolar IC_{50} values has been achieved with certain derivatives of **1**, the challenges for their clinical success remain high. On the other hand, the drugability of the non-mevalonate pathway enzymes in apicoplasts in general and specifically in *Plasmodium* spp. has received additional support by recent findings. Since it has been shown that the antiplasmodial effect of **1** can be reversed by IPP, the final product of the non-mevalonate pathway, it now appears safe to assume that *Pf*IspC is really the one and only target of **1** in the parasite.[21] It has also been shown that the elimination of the apicoplast is lethal in case of *Toxoplasma gondii*.[19,22] However, whereas inhibition studies with recombinant *Pf*IspC can be used for the fine-tuning of the inhibitor-target interaction, the progression of chemically modified derivatives of **1** to the clinical level depends crucially on the combination of the enzyme-based analysis with other preclinical tools.

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. IR spectra were recorded on a Varian 800 FT-IR Scimitar series. 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. If necessary, the purity was determined by HPLC. Analytical high-pressure liquid chromatography (HPLC) was performed in analogy to a previously reported procedure.[23] Instrument: Elite LaChrom system [Hitachi L-2130 (pump) and L-2400 (UV-detector)]; column: 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 determined by HPLC was 95% or higher.

Experimental Data for Compounds. Experimental data are listed below for selected compounds **4e**, **9e**, **11e**, **13**, and **14**.

General Procedure for the Synthesis of *O*-Bn-Protected Hydroxamic Acids 9a−h, 10a−c. To a solution of the respective carboxylic acid **8a−g** (1 equiv, 5 mmol) in dry dichloromethane (20 mL), 1,1′-carbonyldiimidazole (1.1 equiv, 5.5 mmol, 0.89 g) was added in small portions. After stirring 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), once with a saturated aqueous solution of $NaHCO_3$ (10 mL), and once with water (10 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Hydroxamic acids **9a−k** and **10a−c** were purified by column chromatography on silica gel using ethyl acetate as the eluent.

Diethyl ((2-((Benzyloxy)(methyl)amino)-2-oxoethoxy)(3,4-difluorophenyl)methyl)-phosphonate (9e). Yellow oil (6.11 g, 89%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ = 1.25 (t, J = 7.1 Hz, 3H, $POCH_2CH_3$), 1.28 (t, J = 7.1 Hz, $POCH_2CH_3$), 3.19 (s, 3H, NCH_3), 3.96 (d, J = 16.2 Hz, 1H, OCH_2C=O), 4.05−4.18 (m, 4H, $POCH_2CH_3$), 4.33 (dd, J_1 = 16.1 Hz, J_2 = 1.0 Hz, 1H, OCH_2C=O), 4.72 (dd, J_1 = 10.8 Hz, J_2 = 9.4 Hz, 2H, OCH_2Ph), 4.91 (d, J = 14.7 Hz, 1H, PC*H*), 7.11−7.19 (m, 2H), 7.22−7.28 (m, 3H), 7.22−7.35 (m, 3H) ppm; ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 16.36 (d, $^3J_{C,P}$ = 6.6 Hz, $POCH_2CH_3$), 16.41 (d, $^3J_{C,P}$ = 6.5 Hz, $POCH_2CH_3$), 33.45 (NCH_3), 63.20 (d, $^2J_{C,P}$ = 6.9 Hz, $POCH_2CH_3$), 63.46 (d, $^2J_{C,P}$ = 6.4 Hz, $POCH_2CH_3$), 66.37 (d, $^3J_{C,P}$ = 14.1 Hz, OCH_2C=O), 76.25 (OCH_2Ph), 76.82 (d, $^1J_{C,P}$ = 167.8 Hz, PCH), 117.21 (dd, $^2J_{C,F}$ = 17.4 Hz, $^3J_{C,F}$ = 1.7 Hz), 117.29 (dd, $^2J_{C,F}$ = 18.0 Hz, $^3J_{C,F}$ = 5.3 Hz), 124.50 (ddd, $^3J_{C,P}$ = $^3J_{C,F}$ = 6.1 Hz, $^4J_{C,F}$ = 3.6 Hz), 128.73 (2C), 129.21, 129.37 (2C), 131.34 (m), 133.91, 150.32 (ddd, $^1J_{C,F}$ = 249.0 Hz, $^2J_{C,F}$ = 15.1 Hz, $^4J_{C,P}$ = 3.0 Hz), 150.43 (ddd, $^1J_{C,F}$ = 248.9 Hz, $^2J_{C,F}$ = 12.3 Hz, $^5J_{C,P}$ = 2.7 Hz), 170.90 (C=O) ppm; IR (NaCl): $\tilde{\nu}$ = 2984 ($C-H_{aliph.}$), 1682 (C=O), 1285, 1253 (P=O), 1051, 1028 (P−O) cm^{-1}; Anal. Calcd. for $C_{21}H_{26}F_2NO_6P$: C, 55.14; H, 5.73; N, 3.06, found: C, 54.90; H, 5.66; N, 3.08.

General Procedure for the Synthesis of Carboxylic Acids 8a−g, Hydroxamic Aids 11a−h, Target Compounds 4i−k, and Prodrug 14. Benzyl esters **7a−g**, Bn-protected compounds **9a−h**, **12a−c**, and Bn-protected prodrug **13** (1 mmol) were dissolved in methanol (30 mL), respectively. In cases of **7f** and **11f**, ethyl acetate was used as a solvent. Pd−C catalyst (10%, 15% w/w) was added and the respective mixture was hydrogenated for 2 h at atmospheric pressure. The catalyst was removed by filtration and the solvent was evaporated in vacuo to yield crystalline compounds **8a,b,d,e,g**, **11a−e,g**, and **4i**. Carboxylic acids **8c,f** and hydroxamic acids **11f,h** and **14** were obtained as colorless or yellow oils. In the case of target compounds **4j,k**, catalytic hydrogenation yielded hygroscopic resins.

Diethyl ((3,4-Difluorophenyl)(2-(hydroxy(methyl)amino)-2-oxoethoxy)methyl)-phosphonate (11e). Yellow oil (1.84 g, 99%); ^{1}H NMR (500.13 MHz, [D_6]DMSO): δ = 1.26 (t, J = 7.2 Hz, 3H, $POCH_2CH_3$), 1.29 (t, J = 7.1 Hz, $POCH_2CH_3$), 3.27 (s, 3H, NCH_3), 4.05−4.20 (m, 4H, $POCH_2CH_3$), 4.12 (d, J = 14.6 Hz, 1H, OCH_2C=O), 4.61 (d, J = 14.6 Hz, 1H, OCH_2C=O), 4.89 (d, J = 15.9 Hz, 1H, PC*H*), 7.08−7.29 (m, 3H), 9.36 (br.s, 1H, O*H*) ppm; ^{13}C NMR (125.76 MHz, [D_6]DMSO): δ = 16.31 (d, $^3J_{C,P}$ = 5.6 Hz, $POCH_2CH_3$), 16.33 (d, $^3J_{C,P}$ = 5.4 Hz, $POCH_2CH_3$), 35.99 (NCH_3), 63.66 (d, $^2J_{C,P}$ = 7.2 Hz, $POCH_2CH_3$), 64.17 (d, $^2J_{C,P}$ = 6.7 Hz, $POCH_2CH_3$), 68.42 (d, $^3J_{C,P}$ = 13.5 Hz, OCH_2C=O), 76.84 (d, $^1J_{C,P}$ = 170.9 Hz, PCH), 116.83 (dd, $^2J_{C,F}$ = 18.4 Hz, $^3J_{C,F}$ = 4.7 Hz), 117.37 (dd, $^2J_{C,F}$ = 17.3 Hz, $^3J_{C,F}$ = 1.1 Hz), 124.09 (m), 131.04 (m), 150.38 (ddd, $^1J_{C,F}$ = 248.5 Hz, $^2J_{C,F}$ = 12.7 Hz, $^5J_{C,P}$ = 2.1 Hz), 150.48 (ddd, $^1J_{C,F}$ = 248.5 Hz, $^2J_{C,F}$ = 12.5 Hz, $^4J_{C,P}$ = 2.5 Hz), 169.36 (C=O) ppm; IR (NaCl): $\tilde{\nu}$ = 3154 (O−H), 2985, 2913 ($C-H_{aliph.}$), 1665 (C=O), 1286 (P=O), 1050, 1028 (P−O) cm^{-1}; Anal. Calcd. for $C_{14}H_{20}F_2NO_6P$: C, 45.78; H, 5.49; N, 3.81; found: C, 46.00; H, 5.38; N, 3.68.

((((3,4-Difluorophenyl)(2-(hydroxy(methyl)amino)-2-oxoethoxy)methyl)phosphoryl)bis(oxy))-bis(methylene) Bis-(2,2-dimethylpropanoate) (14). Yellow oil (0.27 g, 99%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ = 1.20, 1.22 (2s, 18H, $C(CH_3)_3$), 3.26 (s, 3H, NCH_3), 4.16 (d, J = 14.5 Hz, 1H, OCH_2C=O), 4.57 (d, J = 14.5 Hz, 1H, OCH_2C=O), 4.95 (d, J = 15.2 Hz, 1H, PC*H*), 5.58−5.73 (m, 4H, $POCH_2$), 7.09−7.20 (m, 2H), 7.22−7.32 (m, 1H), 8.74 (br.s, 1H, O*H*) ppm; ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 26.76, 27.07 (2s, $C(CH_3)_3$), 36.04 (NCH_3), 38.72 ($C(CH_3)_3$), 68.68 (d, $^3J_{C,P}$ = 12.9 Hz, OCH_2C=O), 76.59 (d, $^1J_{C,P}$ = 163.1 Hz, PCH), 82.28 (d, $^2J_{C,P}$ = 6.6 Hz, $POCH_2$), 82.36 (d, $^2J_{C,P}$ = 7.2 Hz, $POCH_2$), 117.10 (dd, $^2J_{C,F}$ = 18.5 Hz, $^3J_{C,F}$ = 5.6 Hz), 117.50 (d, $^2J_{C,F}$ = 16.7 Hz), 124.35 (m), 129.86 (m), 150.39 (dd, $^1J_{C,F}$ = 245.8 Hz, $^2J_{C,F}$ = 11.4 Hz), 150.70 (dd, $^1J_{C,F}$ = 249.4 Hz, $^2J_{C,F}$ = 12.1 Hz), 169.06 (*C*=O, hydroxamate), 176.81 (*C*=O, ester), 176.90 (*C*=O, ester) ppm; IR (KBr): $\tilde{\nu}$ = 3196 (O−H), 2978 ($C-H_{aliph.}$), 1756 (C=O, ester), 1666 (C=O), 1283 (P=O), 1024, 1007 (P−O) cm^{-1}; Anal. Calcd. for $C_{22}H_{32}F_2NO_{10}P$: C, 48.98; H, 5.98; N, 2.60; found: C, 49.19; H, 6.17; N, 2.51.

General Procedure for the Synthesis of Target Compounds 4a−h. To a solution of the respective phosphonic acid diethyl ester **11a−h** (1 equiv, 3 mmol) in dry dichloromethane (10 mL), trimethylsilyl bromide (5 equiv, 15 mmol, 1.99 mL) was added at 0 °C. After 1 h, the solution was allowed to warm up to room temperature and stirred for additional 23 h. The solvent was removed under reduced pressure; the remaining residue was dissolved in THF

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(10 mL), and water (0.1 mL) was added. After 30 min, the solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate (20 mL). The organic layer was washed twice with 1 M hydrochloric acid (2 mL), dried over $MgSO_4$, and concentrated. Phosphonic acids **4a–h** were treated with ethyl acetate and stored at 7 °C to yield pure white solids.

((3,4-Difluorophenyl)(2-(hydroxy(methyl)amino)-2-oxoethoxy)methyl)phosphonic Acid (4e). White solid (0.86 g, 55%); mp: 160 °C; ^{1}H NMR (500.13 MHz, [D$_6$]DMSO): δ = 3.07 (s, 3H, NCH_3), 4.26 (d, J = 16.1 Hz, 1H, OCH_2C=O), 4.36 (d, J = 16.1 Hz, 1H, OCH_2C=O), 4.72 (d, J = 14.1 Hz, 1H, PCH), 7.19–7.25 (m, 1H), 7.34–7.46 (m, 2H), 9.88 (br.s, 1H, OH) ppm; ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): δ = 35.65 (NCH_3), 67.14 (d, $^3J_{C,P}$ = 8.5 Hz, OCH_2C=O), 77.51 (d, $^1J_{C,P}$ = 158.7 Hz, PCH), 116.63 (m), 124.68 (m), 134.42 (m), 148.73 (ddd, $^1J_{C,F}$ = 244.2 Hz, $^2J_{C,F}$ = 12.6 Hz, $^5J_{C,P}$ = 2.6 Hz), 148.89 (ddd, $^1J_{C,F}$ = 244.9 Hz, $^2J_{C,F}$ = 12.6 Hz, $^4J_{C,P}$ = 2.0 Hz), 169.27 (C=O) ppm; IR (KBr): $\tilde{\nu}$ = 3138 (O–H), 2877 (C–H$_{aliph.}$), 1621 (C=O), 1206 (P=O), 1031 (P–O) cm^{-1}; Anal. Calcd. for $C_{10}H_{12}F_2NO_6P$: C, 38.60; H, 3.89; N, 4.50; found: C, 38.85; H, 4.08; N, 4.58.

((((2-((Benzyloxy)(methyl)amino)-2-oxoethoxy)(3,4-difluorophenyl)methyl)phosphoryl)bis(oxy))-bis(methylene) Bis(2,2-dimethylpropanoate) (13). To a solution of protected hydroxamic acid **9e** (1 equiv, 4 mmol, 1.83 g) in dry dichloromethane (20 mL), trimethylsilyl bromide (5 equiv, 20 mmol, 2.64 mL) was added at 0 °C. After 1 h, the solution was allowed to warm up to room temperature and stirred for further 23 h. The solvent was removed under reduced pressure; the residue was dissolved in THF (20 mL) and treated with water (0.2 mL). After 30 min, the solvent was evaporated and the residue was dried in vacuo overnight. The residue was dissolved in anhydrous DMF (15 mL), treated with triethylamine (3 equiv, 12 mmol, 1.66 mL), and after stirring for 10 min at room temperature, chloromethyl pivalate (10 equiv, 40 mmol, 5.80 mL) was added. The solution was heated to 70 °C for 2 h. The mixture was treated again with triethylamine (1 equiv, 4 mmol, 0.55 mL) and chloromethyl pivalate (1.5 equiv, 6 mmol, 0.87 mL) and stirred for further 2 h at 70 °C. The procedure of adding triethylamine and chloromethyl pivalate was repeated once again. After two more hours at 70 °C, the reaction mixture was allowed to cool down to room temperature and stirred overnight. The solution was diluted with diethyl ether (120 mL) and washed with water (60 mL), saturated aqueous solution of $NaHCO_3$ (2 × 60 mL), and once again with water (60 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent evaporated in vacuo. Purification of crude **13** was accomplished by column chromatography on silica gel with diethyl ether as the eluent to yield a yellow oil (0.93 g, 37%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ = 1.20, 1.23 (2s, 18H, C(CH_3)$_3$), 3.18 (s, 3H, NCH_3), 3.99 (d, J = 16.2 Hz, 1H, OCH_2C=O), 4.33 (d, J = 16.1 Hz, 1H, OCH_2C=O), 4.74 (dd, J_1 = 16.3 Hz, J_2 = 10.9 Hz, 2H, CH_2Ph), 4.95 (d, J = 14.2 Hz, 1H, PCH), 5.60–5.73 (m, 4H, POCH_2), 7.11–7.18 (m, 2H), 7.22–7.28 (m, 3H), 7.30–7.35 (m, 3H) ppm; ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 26.79, 27.05 (2s, C(CH_3)$_3$), 33.41 (NCH_3), 38.67 (C(CH_3)$_3$), 66.77 (d, $^3J_{C,P}$ = 11.8 Hz, OCH_2C=O), 76.23 (OCH_2Ph), 76.61 (d, $^1J_{C,P}$ = 158.7 Hz, PCH), 82.13 (d, $^2J_{C,P}$ = 6.3 Hz, POCH_2), 82.41 (d, $^2J_{C,P}$ = 6.5 Hz, POCH_2), 117.37 (m, 2C), 124.56 (m), 128.75 (2C), 129.36, 129.44 (2C), 130.17 (m), 133.88, 150.31 (d, $^1J_{C,F}$ = 251.2 Hz), 150.65 (d, $^1J_{C,F}$ = 249.7 Hz), 170.54 (C=O, hydroxamate), 176.71 (C=O, ester), 176.75 (C=O, ester) ppm; IR (KBr): $\tilde{\nu}$ = 2977 (C–H$_{aliph.}$), 1754 (C=O, ester), 1681 (C=O, hydroxamate), 1281 (P=O), 1025 (P–O) cm^{-1}; Anal. Calcd. for $C_{29}H_{38}F_2NO_{10}P$: C, 55.32; H, 6.08; N, 2.22; found: C, 55.49; H, 6.06; N, 2.17.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, analytical data, enzyme assays, and biological evaluation of *in vitro* antiplasmodial activity. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (+49)21181-14985. Fax: -13847. E-mail: thomas.kurz@uni-duesseldorf.de.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CDI, 1,1′-carbonyldiimidazole; DCC, dicyclohexylcarbodiimide; DEP, diethylphosphite; DMF, *N*,*N*-dimethylformamide; DOXP, 1-deoxy-D-xylulose 5-phosphate; Dxr, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; NADPH, nicotinamide adenine dinucleotide phosphate; Pd–C, palladium on activated carbon; *Pf* IspC, *P. falciparum* IspC; RT, room temperature; SD, standard deviation; TEA, triethylamine; THF, tetrahydrofuran; TMSBr, trimethylsilyl bromide

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