Full Paper

γ-Substituted Bis(pivaloyloxymethyl)ester Analogues of Fosmidomycin and FR900098

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The synthesis and *in-vitro* antimalarial activity of γ-substituted bis(pivaloyloxymethyl)ester analogues of the drug candidate fosmidomycin have been investigated. In contrast to the high antimalarial activity of α-aryl substituted fosmidomycin analogues like α-phenylfosmidomycin, γ-substituted derivatives display only weak to moderate activity against the chloroquine-sensitive strain 3D7 of *Plasmodium falciparum*.

Keywords: Fosmidomycin / Malaria tropica / *Plasmodium falciparum* / γ-Substituted analogues

Received: May 18, 2007; accepted: August 24, 2007

DOI 10.1002/ardp.200700107

Introduction

The discovery of the (1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate) DOXP/MEP pathway of isoprenoid biosynthesis, which is for instance present in the malaria parasite *Plasmodium falciparum* and in several pathogenic bacteria or higher plants but not in humans, has stimulated intensive research activities in medicinal and agricultural chemistry [1]. In *Plasmodium falciparum* the DOXP/MEP pathway is located in a plastid-like organelle termed apicoplast that is surrounded by four membranes [2]. The DOXP/MEP pathway leads to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the common precursors of all isoprenoids [2]. Since the resistance of *Plasmodium falciparum* to established antimalarial drugs steadily increases, new classes of compounds displaying novel modes of action have to be developed. The phosphonohydroxamic acid antibiotics fosmidomycin and FR900098 are potent inhibitors of the 1-deoxy-D-xylulose-5-phosphate-reductoisomerase (DXR), the second enzyme of the DOXP/MEP pathway [3]. Clinical trials conducted with fosmidomycin in combination with clindamycin [4] have already demonstrated high efficiency in the treatment of acute, uncomplicated Malaria tropica. However, a disadvantage of fosmidomycin is its quite poor oral bioavailability of approximately 30% [2]. Recently, Schlitzer *et al*. and our group have shown that the hygroscopicity of phosphonohydroxamic acids can be overcome by masking the phosphonic acid moiety as bis(pivaloyloxymethyl) esters [5, 6].

In order to improve fosmidomycin's **1** (Fig. 1) antimalarial activity and physicochemical properties, various chemical modifications have been realised. The hydroxamic acid group [7], the phosphonic acid moiety [8] and the propyl chain are key structural elements for distinct antimalarial activity [9]. In addition, Schlitzer and Ortmann have demonstrated, that prodrugs of FR900098 **2** (Fig. 1) exhibit improved *in-vivo* antimalarial activity against *Plasmodium vinckei* in the mouse model [6]. Recently, modifications of the carbon spacer have been reported. We have discovered that α-phenylfosmidomycin (**3**, IC_{50}: 0.4 μM; Fig. 1) displays potent antimalarial activity against the chloroquine-resistant strain Dd2 of *Plasmodium falciparum* [10]. Van Calenbergh *et al*. confirmed the high activity of aryl substituted fosmidomycin analogues and also described cyclopropyl analogues with potent activity [11]. In a previous publication [5], we reported that the non-hygroscopic bis(pivaloyloxy-

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Abbreviations: (1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate) DOXP/MEP; 1-deoxy-D-xylulose-5-phosphate-reductoisomerase (DXR); bis(pivaloyloxymethyl) ester analogue (3-POM)

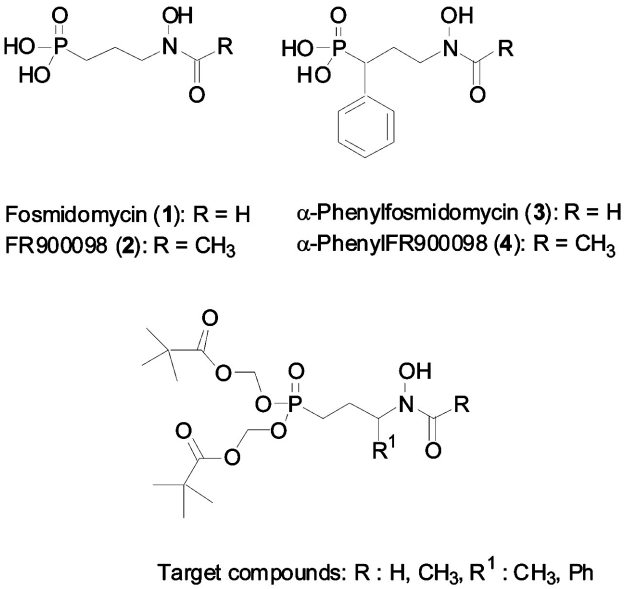


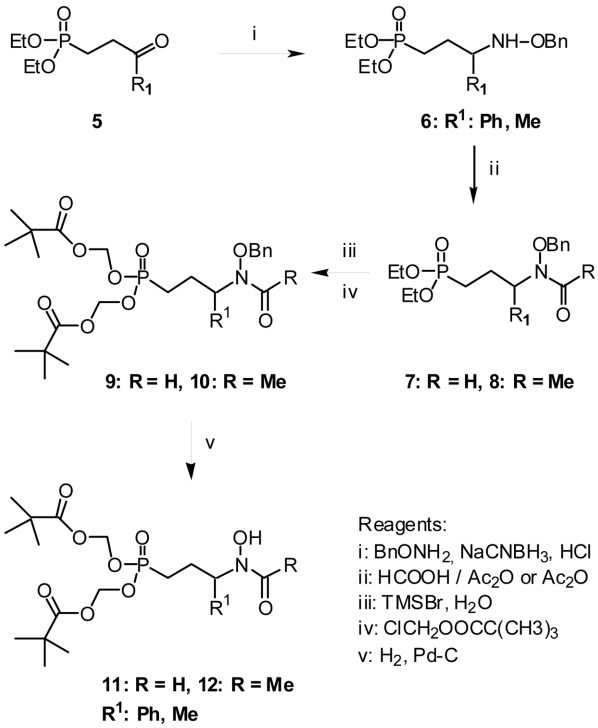
Figure 1. Fosmidomycin and analogues.

methyl) ester analogue (3-POM, Table 1) of α-phenylfosmidomycin **3** displays an IC_{50} value of 0.6 μM towards the chloroquine-sensitive strain 3D7 of *Plasmodium falciparum,* which is in good accordance with the activity of the free acid [5, 10]. However, the most active aryl-analogues reported so far bear a 3,4-dihalophenyl-substituent in the α-position of the propyl-spacer [5, 10, 11]. Furthermore, we have shown that a 3,4-dichlorobenzyl-substituent in the α-position of fosmidomycin's propyl chain leads to high *in-vitro* antimalarial activity against the 3D7 strain (IC_{50} value of 0.9 μM), while the introduction of electron-donating substituents on the phenyl nucleus diminishes the activity [5]. Conformationally restrained bis(pivaloyloxymethyl) ester analogues of **1** and **2** recently investigated exhibit IC_{50} values between 47 – 61 μM against strain 3D7 [12]. In order to obtain further insights of the structure-activity relationships, we now describe the synthesis and *in-vitro* antimalarial activity of γ-substituted bis(pivaloyloxymethyl) ester analogues of the natural products fosmidomycin **1** and FR900098 **2**.

Results and discussion

Chemistry

Since α-substituted analogues of **1** and **2** are accessible from α,β-unsaturated aldehydes [5], we have chosen α,β-unsaturated ketones as starting materials for the preparation of γ-substituted phosphonohydroxamic acids **11** and **12** (Scheme 1). Starting materials **5** were synthesized by treatment of α,β-unsaturated ketones with triethyl



Scheme 1. Synthesis of γ-substituted analogues.

Table 1. Inhibition of *P. falciparum* growth.

Compound	R	R^1	100 μM [%][a]
3-POM	H	H	100
11a	H	Me	38
11b	Me	Me	50
12a	H	Ph	60
12b	Me	Ph	26

[a] Mean values of two independent determinations.

phosphite in ethanol followed by acidic hydrolysis according to a procedure of Harvey [13]. Reactions of **5** with *O*-benzylhydroxylamine and subsequent reduction of the resulting oximes with sodium cyanoborohydride in presence of hydrochloric acid provided *O*-benzyl protected hydroxylamines **6**. Acetylation and formylation of **6** furnished aceto- and formohydroxamic acids (**7** and **8**). Transformation of compounds **7** and **8** into the corresponding bis(pivaloyloxymethyl) esters (**9** and **10**) was accomplished by ester cleavage with trimethylsilyl bromide and subsequent alkylation with chloromethyl pivalate in DMF using triethylamine as a base. Finally, catalytic hydrogenation afforded the target compounds **11**, **12** in almost quantitative yields.

Biological activity

The *in-vitro* antimalarial activity of compounds **11a**, **b** and **12a**, **b** was determined by an 8-[^{3}H]hypoxanthine

incorporation assay [14] using the chloroquine-sensitive strain 3D7 of *Plasmodium falciparum*. Inhibition of parasite growth at 100 μM has been evaluated. The bis(pivaloyloxymethyl) ester of α-phenylfosmidomycin **3** was used as reference compound (Table 1: **3-POM**). All bis(pivaloyloxymethyl) esters were converted into phosphonic acids by non-specific esterases during the experiments.

The *in-vitro* studies have demonstrated that the introduction of a methyl group as well as a phenyl substituent in the γ-position of the propyl chain of **1** and **2** led to a significant reduction of antimalarial activity. Only incomplete inhibition of *Plasmodium falciparum* growth was detected at a concentration of 100 μM (Table 1). The low activity may be due to a sterically hindered coordination of the divalent cation in the active site of the DXR by the hydroxamic acid functionality of compounds **11** and **12**.

Conclusions

This paper describes the synthesis and *in-vitro* antimalarial activity of stable and non-hygroscopic, γ-substituted bis(pivaloyloxymethyl) ester analogues of fosmidomycin **1** and FR900098 **2** and contributes to ongoing structure-activity studies. In contrast to α-aryl substituted analogues, which display high activity, γ-substituted derivatives possess only weak antimalarial activity. The bis(pivaloyloxymethyl) esters are converted into the corresponding acids by non-specific plasma esterases during the determination as shown in previous publications.

The authors have declared no conflict of interest.

Experimental

Elemental analysis was carried out with a Heraeus CHN-O-Rapid instrument (W.C. Heraeus, Hanau, Germany). IR spectra were recorded on a Shimadzu FT-IR 8300 (Shimadzu, Tokyo, Japan). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker AMX 400 spectrometer (Bruker Bioscience, Billerica, MA, USA) using tetramethylsilane as internal standard and DMSO-d_6 or $CDCl_3$ as solvents. Mass spectra were recorded on a Micromass VG 70-250S mass spectrometer (HRFAB; Micromass, Manchester, UK), a Finnegan MAT 311 A mass spectrometer (EI; Thermo Electron Corporation, Bremen, Germany) or a Varian MS 1200L mass spectrometer (ESI; Varian Inc., Palo Alto, CA, USA).

Chemistry

*General procedure for the synthesis of compounds **6a, b***

A solution of the respective ketone **5** (30 mmol) in MeOH (20 mL) was reacted with *O*-benzylhydroxylamine (30 mmol) and stirred at 60°C for 1 h. Afterwards, MeOH (430 mL) was added and the resulting solution treated with $NaCNBH_3$ (90 mmol). HCl (37%, 30 mL) was added dropwise under ice cooling over a period of 30 min. The mixture was allowed to warm up to room temperature, followed by treatment with additional $NaCNBH_3$ (20 mmol). After an overall time of 2 h, the solution was concentrated and a solution of KOH (10%) was added under ice cooling until an alkaline pH was detected. The aqueous solution was extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel using EtOAc/MeOH (90 : 10) as an eluent to yield compounds **6a** and **6b** as colourless oils.

*(3-Benzyloxyamino-butyl)-phosphonic acid diethyl ester **6a***

Yield 93%, IR ν_{max} (KBr) cm^{-1}: 3236 (N-H) and 1246 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 7.36-7.25 (m, 5H), 6.44 (d, 1H, *J* = 5.9 Hz), 4.60 (s, 2H), 4.01-3.92 (m, 4H), 2.99-2.90 (m, 1H), 1.79-1.60 (m, 3H), 1.54-1.43 (m, 1H), 1.21 (t, 6H, *J* = 7.1 Hz), 0.96 (d, 3H, *J* = 6.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6), δ (ppm): 138.74, 128.46, 128.36, 127.75, 75.97, 61.15 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 55.53 (d, $^3J_{C\text{-}P}$ = 17.3 Hz), 26.39 (d, $^2J_{C\text{-}P}$ = 4.6 Hz), 21.35 (d, $^1J_{C\text{-}P}$ = 139 9 Hz), 17.50, 16.64 (d, $^3J_{C\text{-}P}$ = 5.6 Hz); ^{31}P-NMR (202 MHz, DMSO-d_6), δ (ppm): 33.7; Found: C 56.91, H 8.45, N 4.33%. $C_{15}H_{26}NO_4P$ requires C 57.13, H 8.31, N 4.44%.

*(3-Benzyloxyamino-3-phenyl-propyl)-phosphonic acid diethyl ester **6b***

Yield 81%, IR ν_{max} (KBr) cm^{-1}: 3234 (N-H) and 1243 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 7.35 – 7.20 (m, 10H), 6.94 (d, 1H, *J* = 6.62 Hz), 4.53 (dd, 2H, J_{AB} = 11.5 Hz), 4.0 – 3.86 (m, 5H), 2.01 – 1.90 (m, 1H), 1.74 – 1.61 (m, 2H), 1.50 – 1.38 (m, 1H), 1.18 (t, 6H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6), δ (ppm): 142.11, 138.49, 128.48, 128.42, 128.40, 127.92, 127.77, 127.49, 75.77, 64.86 (d, $^3J_{C\text{-}P}$ = 17.3 Hz), 61.23 (d, $^2J_{C\text{-}P}$ = 6.6 Hz), 26.56 (d, $^2J_{C\text{-}P}$ = 4.1 Hz), 21.93 (d, $^1J_{C\text{-}P}$ = 140.4 Hz), 16.60 (d, $^3J_{C\text{-}P}$ = 5.6 Hz); ^{31}P-NMR (202 MHz, DMSO-d_6), δ (ppm): 32.7; Found: C 63.38, H 7.40, N 3.38%. $C_{20}H_{28}NO_4P$ requires C 63.65, H 7.48, N 3.71%.

*General procedure for the synthesis of compounds **7a, b***

Formic acid (500 mmol) was treated with acetic acid anhydride (50 mmol) and stirred under exclusion of humidity. After 20 min, the solution was cooled to 0°C, and the respective hydroxylamine **6a, b** (10 mmol), dissolved in dry THF (20 mL), was added dropwise. After 10 min, the mixture was allowed to warm up to room temperature and stirred for another hour. The solution was treated with EtOAc (200 mL) and successively washed with water (3 × 50 mL), with aqueous KOH (0.1 M, 3 × 25 mL) and once again with water. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc as an eluent to yield compound **7a** as a pale yellow and compound **7b** as colourless oil.

*[3-(Benzyloxy-formyl-amino)-butyl]-phosphonic acid diethyl ester **7a***

Yield 96%, IR ν_{max} (KBr) cm^{-1}: 1679 (C=O) and 1243 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 8.51 – 8.01 (m, 1H), 7.46 – 7.36 (m, 5H), 4.96 (s, 2H), 4.33 – 3.75 (m, 5H), 1.93 – 1.53 (m, 4H), 1.24 (d, 3H, *J* = 6.6 Hz), 1.20 (t, 6H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$), δ

(ppm): 164.77, 134.55, 129.27, 129.02, 128.70, 80.82, 61.69 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 53.62, 26.83 (d, $^2J_{C\text{-}P}$ = 3.1 Hz), 22.95 (d, $^1J_{C\text{-}P}$ = 145.98 Hz), 18.38, 16.44 (d, $^3J_{C\text{-}P}$ = 6.1 Hz); HRFAB-MS $C_{16}H_{26}NO_5P$ MW 343.36, $[M+H]^+$: calculated 344,1627; found 344, 1653.

*[3-(Benzyloxy-formyl-amino)-3-phenyl-propy]-phosphonic acid diethyl ester **7b***

Yield 91%, IR ν_{max} (KBr) cm^{-1}: 1679 (C=O) and 1238 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 8.40 (s, 1H), 7.44 – 7.29 (m, 10H), 5.24 – 4.97 (m, 1H), 4.93 (d, 1H, *J* = 9.2 Hz), 4.61 – 4.46 (m, 1H), 4.03 – 3.93 (m, 4H), 2.41 – 2.30 (m, 1H), 2.19 – 2.06 (m, 1H), 1.79 – 1.67 (m, 2H), 1.21 (t, 6H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 164.15, 137.53, 134.39, 129.37, 128.97, 128.86, 128.58, 128.27, 80.14, 61.72 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 59.58, 23.62, 22.78 (d, $^1J_{C\text{-}P}$ = 142.4 Hz), 16.43 (d, $^3J_{C\text{-}P}$ = 6.1 Hz); Found: C 61.86, H 7.00, N 3.56%. $C_{21}H_{28}NO_5P$ requires C 62.21, H 6.96, N 3.45%.

*General procedure for the synthesis of compounds **8a, b***

Acetic acid anhydride (20 mmol) was added to a solution of the respective hydroxylamine **6a, b** (10 mmol) in dry THF (10 mL) and stirred at room temperature for 2 h. After addition of EtOAc (100 mL), the organic layer was washed with aqueous KOH (0.1 M, 2 × 50 mL), water (50 mL) and with aqueous HCl (1 M, 3 × 20 mL). The organic layer was dried over $MgSO_4$, evaporated, and the residue was purified by column chromatography on silica gel with EtOAc as an eluent to yield compound **8a** as a pale yellow and compound **8b** as colourless oil.

*[3-(Acetyl-benzyloxy-amino)-butyl]-phosphonic acid diethyl ester **8a***

Yield 85%, IR ν_{max} (KBr) cm^{-1}: 1665 (C=O) and 1246 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 7.46 – 7.36 (m, 5H), 4.93 (dd, 2H, $J_{A\text{-}B}$ = 22.1 Hz), 4.39 – 4.21 (m, 1H), 4.01 – 3.91 (m, 4H), 2.11 (s, 3H), 1.95 – 1.83 (m, 1H), 1.79 – 1.61 (m, 3H), 1.21 + 1.20 (2t, 6H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 174.24, 134.59, 128.88, 128.75, 128.70, 79.08, 61.60 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 61.57 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 54.60 (d, $^3J_{C\text{-}P}$ = 16.3 Hz), 27.00 (d, $^2J_{C\text{-}P}$ = 4.6 Hz), 23.02 (d, $^1J_{C\text{-}P}$ = 142.4 Hz), 21.20, 18.47, 16.43 (d, $^3J_{C\text{-}P}$ = 6.1 Hz); HRFAB-MS $C_{17}H_{28}NO_5P$ MW 357.39, $[M+H]^+$: calculated 358.1783; found 358,1773.

*[3-(Acetyl-benzyloxy-amino)-3-phenyl-propyl]-phosphonic acid diethyl ester **8b***

Yield 88%, IR ν_{max} (KBr) cm^{-1}: 1665 (C=O) and 1242 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 7.43 – 7.29 (m, 10H), 5.44 (t, 1H, *J* = 7.7 Hz), 4.81 (d, 1H, *J* = 9.4 Hz), 4.54 (d, 1H, *J* = 9.4 Hz), 4.06 – 3.91 (m, 4H), 2.43 – 2.31 (m, 1H), 2.26 – 2.09 (m, 4H), 1.84 – 1.64 (m, 2H), 1.21 (t, 6H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 173.60, 138.36, 134.34, 128.86, 128.81, 128.69, 128.58, 128.51, 128.21, 78.75, 61.66 (d, $^2J_{C\text{-}P}$ = 7.1 Hz), 60.21 (d, $^3J_{C\text{-}P}$ = 19.3 Hz), 23.40 (d, $^2J_{C\text{-}P}$ = 3.1 Hz), 22.95 (d, $^1J_{C\text{-}P}$ = 142.4 Hz), 21.05, 16.42 (d, $^3J_{C\text{-}P}$ = 6.1 Hz); Found: C 62.75, H 7.17, N 3.27%. $C_{22}H_{30}NO_5P$ calc. C 63.00, H 7.21, N 3.34%.

*General procedure for the synthesis of bis(pivaloyloxymethyl)esters **9a-b, 10a-b***

To a stirred solution of the respective phosphonic acid diethyl esters **7** or **8** (3 mmol) in anhydrous CH_2Cl_2 (20 mL) was added trimethylsilyl bromide (1.5 mL) at 0°C. The solution was stirred at 0°C for one hour and was allowed to warm up to room temperature. After 23 h reaction time, the solvent was evaporated and the residue was dissolved in THF (10 mL). The solution was treated with water (0.1 mL) and stirred for further 5 min before the solvent was removed under reduced pressure to yield the free phosphonic acids.

The resulting crude phosphonic acids were dissolved in anhydrous DMF (20 L), treated with TEA (30 mmol) and chloromethyl pivalate (9 mmol). The mixture was stirred at 70°C for 2 h excluding atmospheric moisture. Another 3 mmol of TEA were added and the solution was stirred over night. The reaction mixture was treated with Et_2O (100 mL) and washed with water (50 mL), with a saturated aqueous $NaHCO_3$-solution (50 mL) and once again with water (50 mL). The organic layer was dried over Na_2SO_4, the solvent evaporated and the residue purified by column chromatography on silica gel with Et_2O as an eluent to yield compounds **9a, b** and **10a, b** as colourless oils.

*2,2-Dimethyl-propionic acid [3-(benzyloxy-formyl-amino)-butyl]-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester **9a***

Yield 34%, IR ν_{max} (KBr) cm^{-1}: 1753 (C=O, ester), 1680 (C=O, hydroxamic acid) and 1252 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 8.32 – 8.04 (m, 1H), 7.47 – 7.33 (m, 5H), 5.69 – 5.63 (m, 4H), 4.97 – 4.86 (m, 2H), 4.40 – 3.72 (m, 1H), 2.05 – 1.73 (m, 4H), 1.32 (m, 3H), 1.22 (s, 18H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.86, 164.80, 134.46, 129.35, 129.09, 128.73, 81.44 (d, $^2J_{C\text{-}P}$ = 5.1 Hz) , 81.38 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 80.90, 53.25, 38.73, 26.85, 26.33, 23.84 (d, $^1J_{C\text{-}P}$ = 141.4 Hz), 18.28; ESI-MS $C_{24}H_{38}NO_9P$, MW 515.55, $[M+Na]^+$: calculated 538; found 538.

*2,2-Dimethyl-propionic acid [3-(benzyloxy-formyl-amino)-3-phenyl-propyl]-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester **9b***

Yield 27%, IR ν_{max} (KBr) cm^{-1}: 1753 (C=O, ester), 1680 (C=O, hydroxamic acid) and 1256 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 8.23 (s, 1H), 7.47 – 7.30 (m, 8H), 7.23 – 7.13 (m, 2H), 5.67 (d, 4H, $^3J_{H\text{-}P}$ = 13 Hz), 5.50 – 5.20 (m, 1H), 4.60 – 4.31 (m, 2H), 2.55 – 2.28 (m, 2H), 1.94 – 1.72 (m, 2H), 1.19 + 1.18 (2s, 18H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.87, 176.84, 163.97, 137.11, 134.32, 129.45, 129.00, 128.92, 128.69, 128.62, 128.32, 81.45 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 79.96, 59.34, 38.70, 26.81, 23.82 (d, $^1J_{C\text{-}P}$ = 142.4 Hz), 23.23; ESI-MS $C_{29}H_{40}NO_9P$, MW 577.62, $[M+H]^+$: calculated 578; found 578.

*2,2-Dimethyl-propionic acid [3-(acetyl-benzyloxy-amino)-butyl]-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester **10a***

Yield 41%, IR ν_{max} (KBr) cm^{-1}: 1752 (C=O, ester), 1668 (C=O, hydroxamic acid) and 1261 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 7.45 – 7.37 (m, 5H), 5.63 – 5.55 (m, 4H), 4.91 (dd, 2H, J_{AB} = 19.7 Hz), 4.36 – 4.23 (m, 1H), 2.10 (s, 3H), 1.89 – 1.78 (m, 3H), 1.73 – 1.62 (m, 1H), 1.21 (d, 3H, *J* = 6.9 Hz), 1.14 (s, 18H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.86, 174.28, 134.46, 128.93, 128.81, 128.74, 82.42 (d, $^2J_{C\text{-}P}$ = 7.1 Hz), 81.35 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 79.21, 54.28 (d, $^3J_{C\text{-}P}$ = 21.4 Hz), 38.71, 26.84, 26.49 (d, $^2J_{C\text{-}P}$ = 4.1 Hz), 23.94 (d, $^1J_{C\text{-}P}$ = 141.4 Hz), 21.19, 18.38; MW 529.57, Anal. Calc. for $C_{25}H_{40}NO_9P$: C 56.70, H 7.61, N 2.64%. Found: C 56.48, H 7.72, N 2.77%.

2,2-Dimethyl-propionic acid [3-(acetyl-benzyloxy-amino)-3-phenyl-propyl]-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester ***10b***

Yield 60%, IR ν_{max} (KBr) cm^{-1}: 1753 (C=O, ester), 1667 (C=O, hydroxamic acid) and 1257 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 7.41 – 7.30 (m, 10H), 5.64 – 5.56 (m, 4H), 5.41 (t, 1H, *J* = 7.6 Hz), 4.78 (d, 1H, *J* = 9.7 Hz), 4.48 (d, 1H, *J* = 9.7 Hz), 2.37 – 2.16 (m, 2H), 2.13 (s, 3H), 1.99 – 1.73 (m, 2H), 1.12 (s, 9H), 1.10 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.83, 173.56, 137.84, 134.25, 128.94, 128.84, 128.76, 128.60, 128.59, 128.33, 81.42 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 78.83, 60.03 (d, $^3J_{C\text{-}P}$ = 20.3 Hz), 38.70, 38.67, 26.84, 26.81, 24.01 (d, $^1J_{C\text{-}P}$ = 142.4 Hz), 22.95 (d, $^2J_{C\text{-}P}$ = 3.6 Hz), 21.05; HRFAB-MS $C_{30}H_{42}NO_9P$, MW 591.64, $[M+H]^+$: calculated 592.2675; found 592.2653.

General procedure for the synthesis of compounds ***11a, b*** *and* ***12a, b***

The respective *O*-benzyl protected hydroxamic acids **9a, b** and **10a, b** (3 mmol) were dissolved in freshly distilled MeOH (50 mL). A catalytic amount of Pd/C was added before the mixture was hydrogenated under 3 bar for one hour. To remove the catalyst, the suspension was filtered through a SPE tube RP-18 purchased from Supelco (Sigma-Aldrich Chemie GmbH, Munich, Germany). The filtrate was evaporated to give the free hydroxamic acids **11a, b** and **12a, b**.

2,2-Dimethyl-propionic acid (2,2-dimethyl-propionyloxy-methoxy)-[3-(formyl-hydroxy-amino)-butyl]-phosphinoyloxymethyl ester ***11a***

Yield 99% (pale yellow oil), IR ν_{max} (KBr) cm^{-1}: 1753 (C=O, ester), 1669 (C=O, hydroxamic acid) and 1239 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 8.54 (s, 1H), 7.95 (s, 1H), 5.70 – 5.59 (m, 4H), 4.47 – 4.39 (m, 0.5H), 3.88 – 3.79 (s, 0.5H), 2.16 – 1.72 (m, 4H), 1.26 – 1.22 (s, 21H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.98, 176.95, 163.77, 81.62 (d, $^2J_{C\text{-}P}$ = 7.1 Hz), 81.50 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 51.27 (d, $^3J_{C\text{-}P}$ = 7.1 Hz), 38.76, 26.86, 26.82, 24.15 (d, $^2J_{C\text{-}P}$ = 5.1 Hz), 22.60 (d, $^1J_{C\text{-}P}$ = 140.4 Hz), 17.36; MW 425.42, Anal. Calc. for $C_{17}H_{32}NO_9P$: C 48.00, H 7.58, N 3.29%. Found: C 47.53, H 7.71, N 3.39%.

2,2-Diemthyl-propionic acid (2,2-dimethyl-propionyloxymethoxy)-[3-(formyl-hydroxy-amino)-3-phenyl-propyl]-phosphinoyloxymethyl ester ***11b***

Yield 99% (pale yellow solid), IR ν_{max} (KBr) cm^{-1}: 1753 (C=O, ester), 1672 (C=O, hydroxamic acid) and 1257 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 8.48 (s, 1H), 8.09 (s, 1H), 7.41 – 7.28 (m, 5H), 5.72 – 5.60 (m, 4H), 5.47 – 5.40 (m, 0.5H), 4.83 – 4.75 (m, 0.5H), 2.64 – 2.43 (m, 1H), 2.28 – 1.80 (m, 3H), 1.24 – 1.20 (m, 18H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.98, 176.94, 163.83, 138.10, 128.84, 128.55, 128.10, 127.58, 127.07, 81.46 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 58.51 (d, $^3J_{C\text{-}P}$ = 10.2 Hz), 38.76, 26.83, 23.19 (d, $^1J_{C\text{-}P}$ = 143.4 Hz), 22.38 (d, $^2J_{C\text{-}P}$ = 4.1 Hz); ESI-MS $C_{22}H_{34}NO_9P$, MW 487.49, $[M+Na]^+$: calculated 510; found 510.

2,2-Dimethyl-propionic acid [3-(acetyl-hydroxy-amino)-butyl]-(2,2dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester ***12a***

Yield 99% (colourless oil), IR ν_{max} (KBr) cm^{-1}: 1754 (C=O, ester), 1618 (C=O, hydroxamic acid) and 1255 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 9.41 (s, 1H), 5.62-5.55 (m, 4H), 4.46 – 4.36 (m, 1H), 1.97 (s, 3H), 1.84 – 1.48 (m, 4H), 1.17 (s, 18H), 1.03 (d, 3H, *J* = 6.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.95, 176.88, 173.05, 81.56 (d, $^2J_{C\text{-}P}$ = 6.1 Hz), 51.39 (d, $^3J_{C\text{-}P}$ = 8.1 Hz), 38.78, 38.75, 26.86, 26.83, 24.36 (d, $^2J_{C\text{-}P}$ = 5.1 Hz), 22.64 (d, $^1J_{C\text{-}P}$ = 140.4 Hz), 20.87, 17.25; MW 439.45, Anal. Calc. for $C_{18}H_{34}NO_9P$: C 49.20, H 7.80, N 3.19%. Found: C 48.60, H 8.00, N 3.08%.

2,2-Dimethyl-propionic acid [3-(acetyl-hydroxy-amino)-3-phenyl-propyl]-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester ***12b***

Yield 99% (colourless oil), IR ν_{max} (KBr) cm^{-1}: 1754 (C=O, ester), 1618 (C=O, hydroxamic acid) and 1254 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 9.66 (s, 1H), 7.35 – 7.25 (m, 5H), 5.63 – 5.56 (m, 4H), 5.49 – 5.42 (m, 1H), 2.19 – 2.11 (m, 1H), 2.03-1.89 (m, 5H), 1.78 – 1.61 (m, 1H), 1.14+1.13 (2s, 18H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 176.95, 176.89, 173.26, 138.72, 128.45, 127.84, 127.61, 81.60 (d, $^2J_{C\text{-}P}$ = 5.1 Hz), 58.52 (d, $^3J_{C\text{-}P}$ = 9.2 Hz), 38.75, 26.83, 23.05 (d, $^1J_{C\text{-}P}$ = 140.4 Hz), 22.43, 20.87; MW 501.52, Anal. Calc. for $C_{23}H_{36}NO_9P$: C 55.08, H 7.24, N 2.79%. Found: C 54.59, H 7.32, N 2.93%.

Determination of *in-vitro* antimalarial activity

Culture of P. falciparum

The *P. falciparum* 3D7 strain was maintained in continuous culture, according to Trager and Jensen and DasGupta *et al*. [15]. The parasites were grown in human red blood cells (RBCs blood group A positive), RPMI 1640 medium supplemented with 25 mM HEPES, 20 mM sodium bicarbonate and 0.5% AlbuMAX (Invitrogen, Karlsruhe, Germany) at 5% hematocrit. The flasks were gassed with 90% N_2, 5% O_2 and 5% CO_2 and incubated at 37°C. The development of the cultures and the percentage of infected RBC`s were determined by light microscopy of Giemsa-stained thin smears.

Preparation of drug solutions

There were 20 µmol of the respective compounds dissolved in 400 µL DMSO and further diluted with water/ethanol (50 / 50) to obtain the particular concentration.

Determination of parasite growth inhibition

The tests were carried out in 96-well microtiter plates under strict aseptic conditions, according to DasGupta *et al*. [15]. Dilutions of each compound were added to 250 µL of a suspension of *P. falciparum* infected erythrocytes (1.5% hematocrit, 1.5 – 2% parasitemia). The plates were flushed with a gas mixture consisting of 90% N_2, 5% O_2 and 5% CO_2, closed tightly and incubated at 37°C for 24 h. Afterwards, 0.1 µCi of 8-[^{3}H]-hypoxanthine was added to each well. The plates were flushed with the above mentioned gas mixture, incubated for additional 24 h at 37°C and subsequently harvested with a cell harvester system (Inotech, Dottikon, Switzerland). Infected erythrocytes were washed four times with distilled water before they were analysed for incorporated radioactivity in a multidetector liquid scintillation counter (Wallac, Turku, Finland).

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