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Conformationally Restrained Aromatic Analogues of Fosmidomycin and FR900098

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The synthesis and *in-vitro* antimalarial activity of conformationally restrained bis(pivaloyloxymethyl) ester analogues of the natural product fosmidomycin is presented. In contrast to α-aryl-substituted analogues, conformationally restrained aromatic analogues exhibit only moderate *in-vitro* antimalarial activity against the chloroquine-sensitive strain 3D7 of *Plasmodium falciparum*. The most active derivative displays an IC_{50} value of 47 μM.

Keywords: Conformationally Restrained Analogues / Fosmidomycin / Malaria tropica / *Plasmodium falciparum*

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Introduction

Every year approximately 1.5 to 2.7 million people die of malaria worldwide. *Plasmodium falciparum*, the causative agent of *malaria tropica*, is responsible for most of the fatal malaria cases. About 90% of the estimated 300 – 500 million malaria cases occur in Sub-Saharan Africa [1]. The widespread resistance of *Plasmodium falciparum* against currently used antimalarial drugs complicates an efficient malaria therapy. Therefore, novel drugs based on new modes of action are urgently needed. A promising organelle for the development of new antimalarias represents the apicoplast of *Plasmodium falciparum* [2]. The plastid-derived apicoplast harbours the DOXP/MEP pathway of isoprenoid biosynthesis, which leads to isopentenyldiphosphate (IPP), an important isoprenoid precursor. Since the DOXP/MEP pathway is absent in humans but present in *Plasmodium falciparum,* several pathogenic bacteria and higher plants, it is an important target for drug development [3].

In 1998, Jomaa discovered that the antibiotic fosmidomycin **1** (Fig. 1) displays its antimalarial activity through the inhibition of the DOXP/MEP pathway of isoprenoid synthesis by blocking the 1-desoxy-d-xylulose-5-phosphate-reductoisomerase (DXR), the second enzyme of the non-mevalonate isoprenoid biosynthesis [4]. Later, fosmidomycin's antimalarial activity was confirmed in various clinical trials [5]. Currently, **1** is in clinical phase-II trials in combination with the lincosamide clindamycin [5].

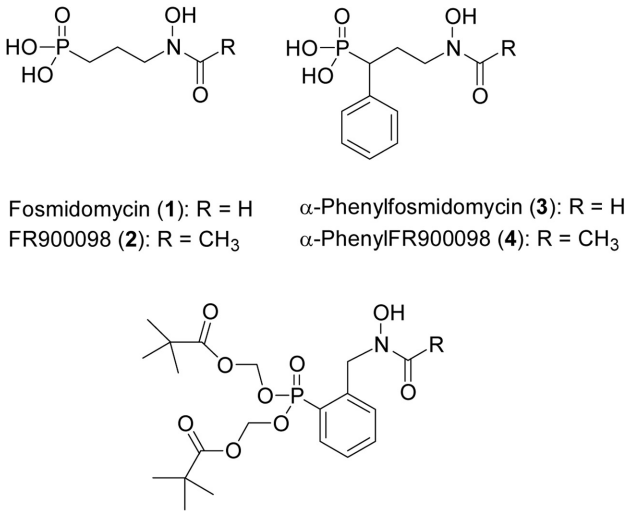

Figure 1. Lead structures and target compounds **1** – **4**.

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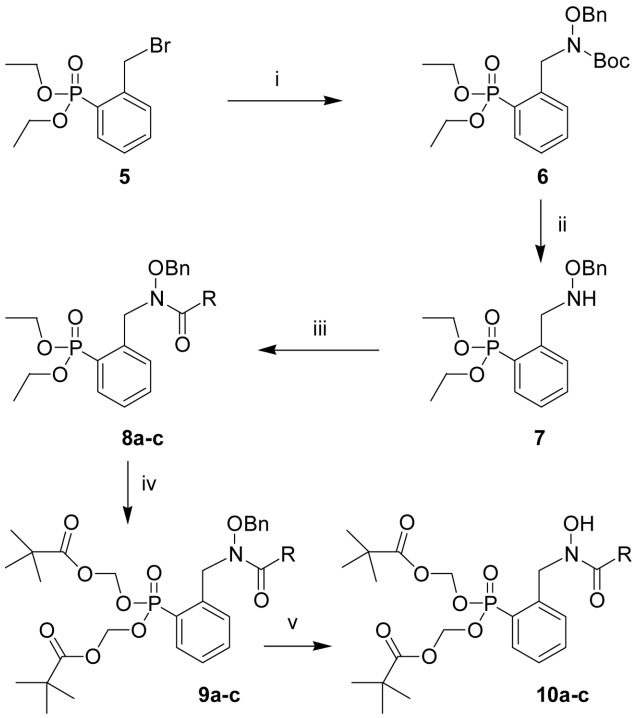
However, a drawback of **1** is its quite low oral bioavailability of approximately 30% [5].

Various groups have contributed to the structural modification of **1** and its more active analogue **2** (Fig. 1). Important structural elements for potent antimalarial activity are the hydroxamic acid functionality [7], the phosphonic acid group [8] and the propyl spacer between both pharmacophores. Improved *in-vivo* antimalarial activity against *Plasmodium vinckei* was observed in case of FR9000098 prodrugs [9]. Recently, modifications of the propyl spacer have been reported. We have shown that α-phenylfosmidomycin (**3**, IC_{50}: 0.4 μM) demonstrates high *in-vitro* activity against the chloroquine-resistant strain Dd2 of *Plasmodium falciparum* [10]. Van Calenbergh *et al.* confirmed our results and also reported on cyclopropane analogues [11]. In addition, Schlitzer and our group have shown that the hygroscopicity of phosphonohydroxamic acids, which limitates their use and analysis, can be overcome by masking the phosphonic acid moiety as bis(pivaloyloxymethyl) (POM) esters [6]. In order to gain new insights regarding the structure-activity relationships, we investigated the synthesis and antimalarial activity of conformationally restrained, aromatic bis(pivaloyloxymethyl) ester analogues of the natural products fosmidomycin **1** and FR900098 **2**.



Reagents. i: NaH, BnONHBoc, ii: TFA / DCM, iii: HOOCH / Ac_2O, or AcCl, Et_3N or BzCl, Et_3N, iv: TMSBr, H_2O, $ClCH_2OOCC(CH_3)_3$, Et_3N, v: H_2, Pd-C, MeOH

Scheme 1. Synthesis of analogues **10a–c**.

Results and discussion

Chemistry

Starting material **5** was synthesized according to a literature procedure [12]. Reaction of **5** with *N*-Boc-*O*-benzylhydroxylamine in presence of sodium hydride provided the protected hydroxycarbamate **6**. Removal of the Boc-protecting group with TFA in dichloromethane and formylation, acetylation or benzoylation of the instable hydroxylamine **7** afforded hydroxamic acids **8a–c**. The conversion of ethyl phosphonates **8a–c** into their corresponding bis(pivaloyloxymethyl) esters **9a–c** was accomplished by treatment with bromotrimethylsilane/water and subsequent alkylation with chloromethyl pivalate in the presence of triethylamine. Finally, catalytic hydrogenation furnished conformationally restrained analogues **10a–c** (Scheme 1).

Biological activity

The *in-vitro* antimalarial activity of compounds **10a–c** was evaluated by an 8-[^{3}H]hypoxanthine incorporation assay [13]. For all experiments the chloroquine-sensitive strain 3D7 of *Plasmodium falciparum* was used. IC_{50} values for compounds **10a–c** have been determined and their antimalarial activity was compared to the bis(pivaloyloxymethyl) esters of fosmidomycin **1**, FR900098 **2** and α-phenylfosmidomycin **3** (see Table 1: **1-POM**, **2-POM**, **3-POM**). During the determination, the bis(pivaloyloxymethyl) esters were converted into free and active phosphonic acids by non-specific esterases.

Table 1. IC_{50} values of bis(pivaloyloxymethyl) ester analogues **10a–c**.

Compound	R	IC_{50} (μM)[a]	n	SEM[b]
10a	Formyl	61	5	7.4
10b	Acetyl	50	6	5.3
10c	Benzoyl	47	6	6.1
1-POM	H	2.1	6	1.1
2-POM	Me	0.4	6	0.1
3-POM	H	0.6	3	0.2

[a] Mean values of three (3) or six (6) independent determinations.

[b] Standard errors of the means.

The modification and rigidisation of fosmidomycin's propyl spacer by a phenyl nucleus led to a significant reduction of antimalarial activity. Analogues **10a–c** display moderate antimalarial activity against the chloroquine-sensitive strain 3D7 in the range of 47–60 μM.

Conclusions

The synthesis and *in-vitro* antimalarial activity of conformationally restrained, aromatic bis(pivaloyloxymethyl) ester analogues of fosmidomycin and FR900098 are described. In contrast to the high antimalarial activity of α-aryl-substituted fosmidomycin analogues as for instance α-phenylfosmidomycin **3**, the aromatic analogues **10a – c** display only moderate *in-vitro* antimalarial activity. Since the bis(pivaloyloxymethyl) esters are transformed into the corresponding phosphonic acids by nonspecific esterases during the determination, no animal experiments were necessary to differentiate between compounds **10a – c**.

Experimental

Elemental analysis was carried out with a Heraeus CHN-O-Rapid instrument (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 (101 MHz) spectra were recorded on a Bruker AMX 400 spectrometer (Bruker, Rheinstetten, Germany) 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).

Chemistry

*{2-[(Benzyloxy-tert-butyloxycarbonyl-amino)-methyl]-phenyl}-phosphonic acid diethyl ester **6***

N-Boc-*O*-benzylhydroxylamine (10mmol) was dissolved in anhydrous THF (30 mL) and the solution was cooled down to 0°C. Under nitrogen atmosphere, sodium hydride (11 mmol) was added portionwise to the stirred solution while the temperature was kept between 0 – 5°C. Ice cooling was removed after gas formation declined. The solution was treated with a catalytic amount of NaI before (2-bromomethyl-phenyl)-phosphonic acid diethyl ester **5** (10 mmol), dissolved in anhydrous THF (5 mL), was added. The reaction mixture was stirred at room temperature over night. Afterwards, ice water was added and the mixture was extracted with diethyl ether (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using diethyl ether/petroleum ether as an eluent to yield compound **6**. Yield 72% (colourless oil); IR ν_{max} (KBr) cm^{-1}: 1705 (C=O), 1252 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$) = δ (ppm) 1.29 (t, *J* = 6.95 Hz, 6H, $POCH_2CH_3$), 1.49 (s, 9H, $(CH_3)_3C$), 3.99 – 4.15 (m, 4H, $POCH_2CH_3$), 4.81 (s, 2H, benzyl. CH_2), 5.03 (s, 2H, benzyl CH_2), 7.27 – 7.37 (m, 6H, aromat.), 7.45 – 7.55 (m, 2H, aromat.), 7.91 – 7.98 (m, 1H, aromat.); ^{13}C-NMR (101 MHz, $CDCl_3$) = δ (ppm) 16.7 (d, $J_{C,P}$ = 6.19 Hz, $POCH_2CH_3$), 28.7 ($(CH_3)_3C$), 51.8 ($(CH_3)_3C$), 62.5 (d, $J_{C,P}$ = 5.54 Hz, $POCH_2CH_3$), 77.4 (benzyl. CH_2), 82.0 (benzyl CH_2), 126.2 (d, $J_{C,P}$ = 183.42 Hz, P*C* quart., aromat.), 127.1 (d, $J_{C,P}$ = 13.73 Hz, tert., aromat.), 128.3 (d, $J_{C,P}$ = 14.02 Hz, tert., aromat.), 128.7, 128.9, 129.8 (tert., aromat.), 133.0 (d, $J_{C,P}$ = 3.04 Hz, PC*C*H tert., aromat.), 134.4, 134.5 (tert., aromat.), 135.8 (quart., aromat.), 141.4 (d, $J_{C,P}$ = 10.36 Hz, PC*C* quart., aromat.), 156.0 (*C*=O); Anal. calcd. for $C_{23}H_{32}NO_6P$: C, 61.46; H, 7.18; N, 3.12. Found: C, 61.20; H, 7.23; N, 3.19.

*{2-(Benzyloxyamino-methyl]-phenyl}-phosphonic acid diethyl ester **7***

A solution of **6** (10 mmol) in dichloromethane (30 mL) was treated with trifluoro acetic acid (10 mL) at 0°C. The solution was stirred at room temperature for 1 h. Afterwards, the solvent was evaporated. The residue was dissolved in a solution of K_2CO_3 (10%, 30 mL) and successively extracted with diethyl ether (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The remaining oil was treated with an aqueous solution of HCl (1 M, 30 mL) and intensively stirred for 15 min before the mixture was extracted with diethyl ether (2 × 30 mL). The aqueous layer was neutralised with a K_2CO_3-solution (10%) and again extracted with diethyl ether (2 × 30 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography on silica gel using diethyl ether / ethyl acetate as an eluent to yield the instable compound **7**. Yield 92% (colourless oil); IR ν_{max} (KBr) cm^{-1}: 3244 (N-H), 1240 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$) = δ (ppm) 1.31 (t, *J* = 6.93 Hz, 6H, $POCH_2CH_3$), 3.99 – 4.21 (m, 4H, $POCH_2CH_3$), 4.32 (s, 2H, benzyl CH_2), 4.76 (s, 2H, benzyl CH_2), 7.25 – 7.41 (m, 6H, aromat.), 7.47 – 7.56 (m, 2H, aromat.), 7.83 – 7.93 (m, 1H, aromat.); ^{13}C-NMR (101 MHz, $CDCl_3$) = δ (ppm) 16.3 (d, $J_{C,P}$ = 6.68 Hz, $POCH_2CH_3$), 54.6 (d, $J_{C,P}$ = 3.74 Hz, benzyl CH_2), 62.3 (d, $J_{C,P}$ = 5.77 Hz, $POCH_2CH_3$), 75.9 (benzyl CH_2), 127.3 (d, $J_{C,P}$ = 183.42 Hz, P*C* quart., aromat.), 127.2 (d, $J_{C,P}$ = 14.59 Hz, tert., aromat.), 127.4, 128.3, 128.4 (tert., aromat.), 132.0 (d, $J_{C,P}$ = 14.72 Hz, tert., aromat.), 132.4 (d, $J_{C,P}$ = 2.72 Hz, PC*C*H tert., aromat.), 133.6, 133.7 (tert., aromat.), 137.9 (quart., aromat.), 140.8 (d, $J_{C,P}$ = 11.32 Hz, P*C*C quart., aromat.). Due to the instability of compound **7** no satisfactory elementary analysis could be obtained. Compound **7** was immediately converted into stable hydroxamic acids **8a – c**.

*{2-[(Benzyloxy-formyl-amino)-methyl]-phenyl}-phosphonic acid diethyl ester **8a***

Acetic acid anhydride (10 mmol) was treated with formic acid (100 mmol) and stirred for 30 min at room temperature excluding humidity. The reaction mixture was cooled to 0°C before a solution of hydroxylamine **7** (1 mmol) in formic acid (5 mL) was added dropwise. The mixture was allowed to warm up to room temperature and was stirred for two more hours. The solution was treated with ethyl acetate (200 mL) and washed with a saturated solution of K_2CO_3 until the aqueous layer reacted alkaline. The organic layer was then washed with water (100 mL) and aqueous HCl (0.5 M, 3 × 100 mL) and dried over $MgSO_4$ The solvent was removed under reduced pressure and the remaining residue was purified by column chromatography on silica gel using diethyl ether/ethyl acetate as an eluent to yield compound **8a**. Yield 97% (pale yellow oil); IR ν_{max} (KBr) cm^{-1}: 1662 (C=O), 1236 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$) = δ (ppm) 1.33 (t, *J* = 7.05 Hz, 6H, $POCH_2CH_3$), 4.01 – 4.26 (m, 4H, $POCH_2CH_3$), 4.84 (s, 2H, benzyl. CH_2), 4.91 – 5.37 (m, 2H, benzyl CH_2), 7.24 – 7.49 (m, 7H, aromat.), 7.50 – 7.60 (m, 1H, aromat.), 7.86 – 7.98 (m, 1H, aromat.), 8.09 – 8.38 (m, 1H, formyl.); ^{13}C-NMR (101 MHz, $CDCl_3$) = δ (ppm) 16.7 (d, $J_{C,P}$ = 6.85 Hz, $POCH_2CH_3$), 46.0 (benzyl. CH_2), 62.8 (d, $J_{C,P}$ = 5.56 Hz, $POCH_2CH_3$), 78.0 (benzyl CH_2), 125.4 (d, $J_{C,P}$ = 134.85 Hz, P*C* quart., aromat.), 127.7 (d, $J_{C,P}$ = 15.77 Hz, tert., aromat.), 129.0 (d, $J_{C,P}$ = 13.65 Hz, tert., aromat.), 129.5, 130.0 (tert.,

aromat.), 134.2 (d, $J_{C,P}$ = 2.99 Hz, PC*C*H tert., aromat.), 134.1, 134.2 (tert., aromat.), 134.6 (quart., aromat.), 139.6 (d, $J_{C,P}$ = 8.52 Hz, PC*C* quart., aromat.), 163.6 (*C*=O); Anal. calcd. for $C_{19}H_{24}NO_5P$: C, 60.47; H, 6.41; N, 3.71. Found: C, 60.42; H, 6.70; N, 3.46.

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

Hydroxylamine **7** (3 mmol) was dissolved in anhydrous dichloromethane (20 mL), treated with triethylamine (4 mmol) and cooled to 0–5°C. Over a period of 10 min the corresponding acid chloride (4 mmol in 5 mL dichloromethane) was added to the stirred solution. The mixture was stirred for 2 h at room temperature and the progress of the reaction was monitored by TLC. The solvent was evaporated, the residue dissolved in ethyl acetate (20 mL) and successively washed with aqueous HCl (1 M, 2 × 20 mL) and with K_2CO_3 solution (10%, 2 × 20 mL). 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 using *n*-hexane/ethyl acetate as an eluent to yield compounds **8b, c**.

*{2-[(Acetyl-benzyloxy-amino)-methyl]-phenyl}-phosphonic acid diethyl ester **8b***

Yield 98% (pale yellow oil); IR ν_{max} (KBr) cm^{-1}: 1668 (C=O), 1246 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$) = δ (ppm) 1.32 (t, *J* = 6.65 Hz, 6H, $POCH_2CH_3$), 2.19 (s, 3H, acetyl. CH_3), 4.02–4.22 (m, 4H, $POCH_2CH_3$), 4.84 (s, 2H, benzyl CH_2), 5.32 (s, 2H, benzyl CH_2), 7.25–7.44 (m, 7H, aromat.), 7.49–7.56 (m, 1H, aromat.), 7.88–7.97 (m, 1H, aromat.); ^{13}C-NMR (101 MHz, $CDCl_3$) = δ (ppm) 16.3 (d, $J_{C,P}$ = 6.23 Hz, $POCH_2CH_3$), 20.5 (acetyl CH_3), 47.1 (benzyl CH_2), 62.3 (d, $J_{C,P}$ = 5.41 Hz, $POCH_2CH_3$), 76.6 (benzyl CH_2), 125.9 (d, $J_{C,P}$ = 132.08 Hz, P*C* quart., aromat.), 126.9 (d, $J_{C,P}$ = 14.76 Hz, tert., aromat.), 128.0 (d, $J_{C,P}$ = 14.76 Hz, tert., aromat.), 128.6, 128.9, 129.3 (tert., aromat.), 132.8 (d, $J_{C,P}$ = 2.84 Hz, PC*C*H tert., aromat.), 133.8, 133.9 (tert., aromat.), 134.3 (quart., aromat.), 140.3 (d, $J_{C,P}$ = 9.65 Hz, PC*C* quart., aromat.), 167.4 (*C*=O); Anal. calcd for $C_{20}H_{26}NO_5P$: C, 61.37; H, 6.70; N, 3.58. Found: C, 61.21; H, 6.69; N, 3.38.

*{2-[(Benzoyl-benzyloxy-amino)-methyl]-phenyl}-phosphonic acid diethyl ester **8c***

Yield 91% (colourless oil); IR ν_{max} (KBr) cm^{-1}: 1651 (C=O), 1246 (P=O); ^{1}H-NMR (400 MHz, $CDCl_3$) = δ (ppm) 1.30 (t, 6H, *J* = 7.34 Hz, $POCH_2CH_3$), 4.01–4.21 (m, 4H, $POCH_2CH_3$), 4.72 (s, 2H, benzyl CH_2), 5.43 (s, 2H, benzyl CH_2), 6.88–7.03 (m, 2H, aromat.), 7.16–7.25 (m, 3H, aromat.), 7.34–7.75 (m, 8H, aromat.), 7.91–7.99 (m, 1H, aromat.); ^{13}C-NMR (101 MHz, $CDCl_3$) = δ (ppm) 16.7 (d, $J_{C,P}$ = 6.07 Hz, $POCH_2CH_3$), 48.8 (benzyl CH_2), 62.7 (d, $J_{C,P}$ = 5.46 Hz, $POCH_2CH_3$), 77.0 (benzyl CH_2), 123.6 (d, $J_{C,P}$ = 183.41 Hz, P*C* quart., aromat.), 127.4 (d, $J_{C,P}$ = 13.96 Hz, tert., aromat.), 128.3 (d, $J_{C,P}$ = 13.40 Hz, tert., aromat.), 128.5, 128.7, 128.8, 129.1, 129.9, 130.4, 131.0 (tert., aromat.), 133.3 (d, $J_{C,P}$ = 2.68 Hz, PC*C*H tert., aromat.), 134.3, 134.4 (tert., aromat.), 134.6 (quart., aromat.), 140.7 (d, $J_{C,P}$ = 8.52 Hz, PC*C* quart., aromat.), 170.7 (*C*=O); Anal. Calcd for $C_{25}H_{28}NO_5P$: C, 66.22; H, 6.22; N, 3.09. Found: C, 66.22; H, 6.43; N, 3.03.

*General procedure for the synthesis of compounds **9a–c***

Trimethylsilyl bromide (6 mmol) was added dropwise to a stirred solution of the respective phosphonic acid diethyl esters **8a–c** (1 mmol) in anhydrous dichloromethane at 0°C via a syringe. The mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure. Afterwards, the remaining residue was dissolved in THF (3 mL), treated with one drop of water and stirred at room temperature. After 10 min, the solvent was evaporated and remaining water was removed *in vacuo* over night. The resulting phosphonic acid was dissolved in anhydrous DMF (10 mL) and treated with TEA (3 mmol) and chloromethyl pivalate (10 mmol). Under anhydrous conditions, the mixture was heated to 70°C and stirred for 2 h. The solution was treated again with TEA (1 mmol) and chloromethyl pivalate (2 mmol) and stirred for further 2 h. Afterwards, the procedure of adding TEA and chloromethyl pivalate was repeated once again. The mixture was stirred for two more hours at 70°C, allowed to cool down to room temperature and finally stirred over night. Diethyl ether (50 mL) was added and the solution was successively washed with water (25 mL), an aqueous saturated $NaHCO_3$-solution (2 × 25 mL) and again with water (25 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica gel using diethyl ether/*n*-hexane as an eluent to yield compounds **9a–c**.

*2,2-Dimethyl-propionic acid{2-[(benzyloxy-formyl-amino)-methyl]-phenyl}-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester **9a***

Yield 33% (colourless oil); IR ν_{max} (KBr) cm^{-1}: 1751 (C=O), 1680 (C=O, hydroxamic acid), 1259 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6) = δ (ppm) 1.02 (s, 18H, $((CH_3)_3C)$, 4.91 (s, 2H, benzyl. CH_2), 5.02 (s, 2H, benzyl CH_2), 5.62–5.82 (m, 4H, OCH_2O), 7.23–7.57 (m, 7H, aromat.), 7.60–7.89 (m, 2H, aromat.), 8.09–8.51 (m, 1H, formyl); ^{13}C-NMR (101 MHz, DMSO-d_6) = δ (ppm) 27.1 ($(CH_3)_3C$), 39.1 ($(CH_3)_3C$), 45.6 (benzyl CH_2), 77.1 (benzyl CH_2), 82.2 (OCH_2O), 124.6 (d, $J_{C,P}$ = 135.32 Hz, P*C* quart., aromat.), 127.7 (d, $J_{C,P}$ = 15.12 Hz, tert., aromat.), 129.1, 129.5, 130.1 (tert., aromat.), 133.9 (d, $J_{C,P}$ = 2.97 Hz, PC*C*H tert., aromat.), 134.1 (d, $J_{C,P}$ = 10.84 Hz, tert., aromat), 134.7 (quart., aromat.), 139.8 (d, $J_{C,P}$ = 10.92 Hz, PC*C* quart., aromat.), 163.5 (*C*=O, hydroxamic acid), 177.2 (*C*=O, acylal); Anal. calcd. for $C_{27}H_{36}NO_9P$: C, 59.01; H, 6.60; N, 2.55. Found: C, 58.79; H, 6.77; N, 2.37.

*2,2-Dimethyl-propionic acid{2-[(acetyl-benzyloxy-amino)-methyl]-phenyl}-(2,2-dimethyl-propionyloxymethoxy)-phophinoyloxymethyl ester **9b***

Yield 37% (colourless oil); IR ν_{max} (KBr) cm^{-1}: 1753 (C=O), 1668 (C=O, hydroxamic acid), 1259 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6) = δ (ppm) 1.02 (s, 18H, $((CH_3)_3C)$, 2.15 (s, 3H, acetyl. CH_3), 4.90 (s, 2H, benzyl CH_2), 5.12 (s, 2H, benzyl CH_2), 5.66–5.80 (m, 4H, OCH_2O), 7.21–7.30 (m, 1H, aromat.), 7.32–7.50 (m, 6H, aromat.), 7.60–7.67 (m, 1H, aromat.), 7.75–7.86 (m, 1H, aromat.); ^{13}C-NMR (101 MHz, DMSO-d_6) = δ (ppm) 20.6 (acetyl CH_3), 26.6 ($(CH_3)_3C$), 38.5 ($(CH_3)_3C$), 46.6 (benzyl CH_2), 76.0 (benzyl CH_2), 82.2 (d, $J_{C,P}$ = 6.04 Hz, OCH_2O), 125.4 (d, $J_{C,P}$ = 185.87 Hz, P*C* quart., aromat.), 127.4 (d, $J_{C,P}$ = 14.69 Hz, tert., aromat.), 127.6 (d, $J_{C,P}$ = 14.70 Hz, tert., aromat.), 128.8, 129.2, 129.9, 133.5, 133.6 (tert., aromat.), 133.9 (d, $J_{C,P}$ = 2.88 Hz, PC*C*H tert., aromat.), 134.7 (quart., aromat.), 140.7 (d, $J_{C,P}$ = 10.30 Hz, PC*C* quart., aromat.), 171.7 (*C*=O, hydroxamic acid), 176.7 (*C*=O, acylal); Anal. calcd. for $C_{28}H_{38}NO_9P$: C, 59.67; H, 6.80; N, 2.49. Found: C, 59.53; H, 6.68; N, 2.51.

2,2-Dimethyl-propionic acid{2-[(benzoyl-benzyloxy-amino)-methyl]-phenyl}-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester **9c**

Yield 41% (colourless oil); IR ν_{max} (KBr) cm^{-1}: 1753 (C=O), 1649 (C=O, hydroxamic acid), 1257 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6) = δ (ppm) 1.02 (s, 18H, ((CH_3)$_3$C), 4.74 (s, 2H, benzyl. CH_2), 5.28 (s, 2H, benzyl CH_2), 5.68 – 5.79 (m, 4H, OCH_2O), 6.90 – 6.98 (m, 2H, aromat.), 7.18 – 7.31 (m, 3H, aromat.), 7.45 – 7.59 (m, 5H, aromat.), 7.61 – 7.74 (m, 3H, aromat.), 7.79 – 7.88 (m, 1H, aromat.); ^{13}C-NMR (101 MHz, DMSO-d_6) = δ (ppm) 26.2 ((*C*H$_3$)$_3$C), 38.0 ((CH$_3$)$_3$*C*), 47.0 (benzyl. *C*H$_2$), 75.5 (benzyl *C*H$_2$), 81.7 (O*C*H$_2$O), 125.1 (d, $J_{C,P}$ = 184.87 Hz, P*C* quart., aromat.), 127.1 (d, $J_{C,P}$ = 14.88 Hz, tert., aromat.), 127.2 (d, $J_{C,P}$ = 14.41 Hz, tert., aromat.), 127.6, 128.0, 128.1, 128.6, 129.2, 130.4, 131.2, 133.3 (tert., aromat.), 133.6 (d, $J_{C,P}$ = 2.56 Hz, PC*C*H tert., aromat.), 134.1 (quart., aromat.), 140.7 (d, $J_{C,P}$ = 11.00 Hz, PC*C* quart., aromat.), 169.4 (*C*=O, hydroxamic acid), 176.7 (*C*=O, acylal); Anal. calcd. for $C_{33}H_{40}NO_9P$: C, 63.35; H, 6.44; N, 2.24. Found: C, 63.05; H, 6.56; N, 2.29.

General procedure for the synthesis of compound **10a – c**

The respective *O*-benzyl protected hydroxamic acids **9a – c** (1 mmol) were hydrogenated in MeOH (50 mL) using catalytic amounts of 10% Pd/C (2 h, 2 bar). Afterwards, the suspension was filtered through a SPE tube RP-18 purchased from Supelco (Sigma-Aldrich, Munich, Germany) in order to remove the catalyst. The filtrate was evaporated to yield the free hydroxamic acids **10a – c**.

2,2-Dimethyl-propionic acid (2,2-dimethyl-propionyloxymethoxy)-{2-[(formyl-hydroxy-amino)-methyl]-phenyl}-phosphinoyloxymethyl ester **10a**

Yield 81% (yellow oil); IR ν_{max} (KBr) cm^{-1}: 3220 (O-H), 1755 (C=O), 1674 (C=O, hydroxamic acid), 1255 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6) = δ (ppm) 0.99 – 1.07 (m, 18H, ((CH_3)$_3$C), 4.55 (m, 0.25H, benzyl CH_2), 4.92 (m, 1.75H, benzyl CH_2), 5.64 – 5.79 (m, 4H, OCH_2O), 7.30 – 7.52 (m, 2H, aromat.), 7.62 – 7.85 (m, 2H, aromat.), 8.10 – 8.22 (m, 0.45H, formyl), 8.39 – 8.52 (m, 0.55H, formyl), 9.83 (s, 0.45H, O*H*), 10.30 (s, 0.55H, O*H*); ^{13}C-NMR (101 MHz, DMSO-d_6) = δ (ppm) 26.7 ((CH$_3$)$_3$C), 38.5 ((CH$_3$)$_3$*C*), 48.4 (benzyl *C*H$_2$), 82.1 (O*C*H$_2$O), 125.4 (d, $J_{C,P}$ = 186.65 Hz, P*C* quart., aromat.), 127.4 (d, $J_{C,P}$ = 13.34 Hz, tert., aromat.), 133.5 (d, $J_{C,P}$ = 13.44 Hz, tert., aromat.), 133.6 (tert., aromat.), 133.8 (d, $J_{C,P}$ = 2.49 Hz, PC*C*H tert., aromat.), 158.6, 161.7, 162.9 (*C*=O, hydroxamic acid), 176.4 (*C*=O, acylal); Anal. Calcd. for $C_{20}H_{30}NO_9P$: C, 52.29; H, 6.58; N, 3.05. Found: C, 52.11; H, 6.87; N, 2.82.

2,2-Dimethyl-propionic acid {2-[(acetyl-hydroxy-amino)-methyl]-phenyl}-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester **10b**

Yield 79% (colourless oil); IR ν_{max} (KBr) cm^{-1}: 3209 (O-H), 1757 (C=O), 1637 (C=O, hydroxamic acid), 1259 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6) = δ (ppm) 1.01 – 1.09 (m, 18H, ((CH_3)$_3$C), 1.95 (m, 0.4H, acetyl CH_3), 2.14 (m, 2.6H, acetyl CH_3), 4.49 – 4.64 (m, 0.2H, benzyl CH_2), 4.86 – 5.08 (m, 1.8H, benzyl CH_2), 5.62 – 5.81 (m, 4H, OCH_2O), 7.25 – 7.51 (m, 2H, aromat.), 7.62 – 7.87 (m, 2H, aromat.), 10.06 (s, 1H, O*H*); ^{13}C-NMR (101 MHz, DMSO-d_6) = δ (ppm) 20.6 (acetyl *C*H$_3$), 26.7 ((*C*H$_3$)$_3$C), 38.5 ((CH$_3$)$_3$*C*), 49.8 (benzyl *C*H$_2$), 82.0 (O*C*H$_2$O), 125.2 (d, $J_{C,P}$ = 184.58 Hz, P*C* quart., aromat.), 127.1 (d, $J_{C,P}$ = 15.86 Hz, tert., aromat.), 127.3 (d, $J_{C,P}$ = 14.09 Hz, tert., aromat.), 133.5, 133.6 (tert., aromat.), 133.7 (d, $J_{C,P}$ = 2.35 Hz, PC*C*H tert., aromat.), 140.7 ($J_{C,P}$ = 10.57 Hz, PC*C* quart., aromat.), 171.5 (*C*=O, hydroxamic acid), 176.3 (*C*=O, acylal); Anal. calcd. for $C_{21}H_{32}NO_9P$: C, 53.27; H, 6.81; N, 2.96. Found: C, 53.65; H, 7.20; N, 2.82.

2,2-Dimethyl-propionic acid {2-[(benzoyl-hydroxy-amino)-methyl]-phenyl}-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl ester **10c**

Yield 86% (yellow oil); IR ν_{max} (KBr) cm^{-1}: 3361 (O-H), 1751 (C=O), 1649 (C=O, hydroxamic acid), 1257 (P=O); ^{1}H-NMR (400 MHz, DMSO-d_6) = δ (ppm) 1.02-1.09 (m, 18H, ((CH_3)$_3$C), 4.72 (d, *J* = 6.37 Hz, 1.4H, benzyl CH_2), 5.43 (s, 0.6H, benzyl CH_2), 5.64 – 5.79 (m, 4H, OCH_2O), 7.38 – 7.95 (m, 9H, aromat.), 8.98 (t, 0.7H, *J* = 5.90 Hz, O*H*), 10.2 (s, 0.3H, O*H*); ^{13}C-NMR (101 MHz, DMSO-d_6) = δ (ppm) 27.1 ((*C*H$_3$)$_3$C), 39.1 ((CH$_3$)$_{3C}$), 42.7, 42.8 (2d, $J_{C,P}$ = 4.50 Hz, benzyl *C*H$_2$), 82.3 (O*C*H$_2$O), 126.2 (d, $J_{C,P}$ = 187.32 Hz, P*C* quart., aromat.), 127.6 (tert. aromat.), 127.9 (d, $J_{C,P}$ = 14.97 Hz, tert., aromat.), 128.8, 131.8 (tert., aromat.), 132.6 (d, $J_{C,P}$ = 15.19 Hz, tert., aromat.), 133.7, 133.8, 134.1 (tert., aromat.), 134.2 (d, $J_{C,P}$ = 3.32 Hz, PC*C*H tert., aromat.), 134.6 (quart., aromat.), 142.9 (d, $J_{C,P}$ = 10.92 Hz, PC*C* quart., aromat.), 167.2 (*C*=O, hydroxamic acid), 177.4 (*C*=O, acylal); HRFAB-MS $C_{26}H_{34}NO_9P$, MW 535.54, $[M+H]^+$ calculated 536.2051; found 536.2043.

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*. [14]. 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

20 µmol of the respective compounds were 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*. [14]. 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, 0.1µCi 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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