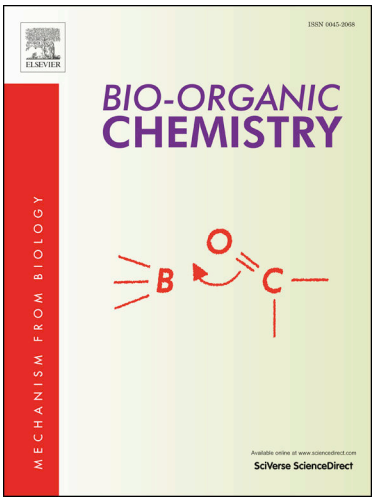
Journal Pre-proofs



Synthesis and Anti-parasitic Activity of Achiral *N*-Benzylated Phosphoramidic Acid Derivatives

Christiana M. Adeyemi, Anne C. Conibear, Marius K. Mutorwa, Iviwe Nokalipa, Michelle Isaacs, Dumisani Mnkandhla, Heinrich C. Hoppe, Kevin A. Lobb, Rosa Klein, Perry T. Kaye

PII: S0045-2068(20)30572-1
DOI: https://doi.org/10.1016/j.bioorg.2020.103947
Reference: YBIOO 103947

To appear in: *Bioorganic Chemistry*

Received Date: 16 March 2020
Revised Date: 11 May 2020
Accepted Date: 14 May 2020

Please cite this article as: C.M. Adeyemi, A.C. Conibear, M.K. Mutorwa, I. Nokalipa, M. Isaacs, D. Mnkandhla, H.C. Hoppe, K.A. Lobb, R. Klein, P.T. Kaye, Synthesis and Anti-parasitic Activity of Achiral *N*-Benzylated Phosphoramidic Acid Derivatives, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.103947

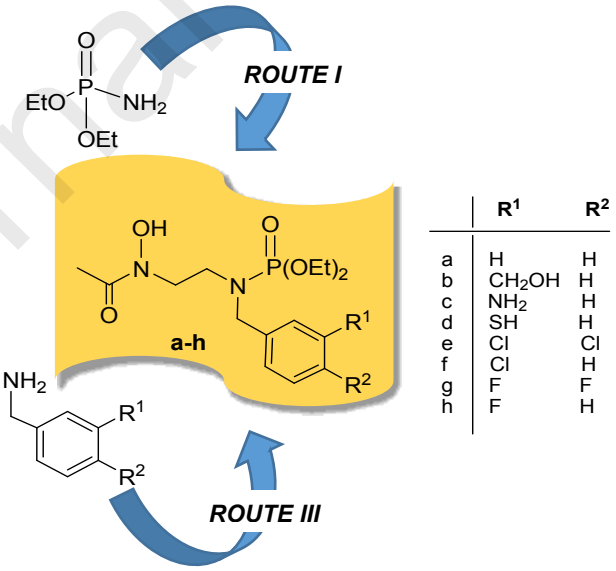
Synthesis and Anti-parasitic Activity of Achiral *N*-Benzylated Phosphoramidic Acid Derivatives

Christiana M. Adeyemi[a], Anne C. Conibear[a†], Marius K. Mutorwa[a‡], Iviwe Nokalipa[a], Michelle Isaacs[c], Dumisani Mnkandhla[c], Heinrich C. Hoppe[b,c], Kevin A. Lobb[a,c], Rosa Klein[a,c], and Perry T. Kaye[a,c*]

[a]Department of Chemistry, [b]Department of Biochemistry and Microbiolgy and [c]Centre for Chemico- and Biomedicinal Research, Rhodes University, Grahamstown, 6140, South Africa.

Abstract. Synthetic pathways have been developed to access a series of *N*-benzylated phosphoramidic acid derivatives as novel, achiral analogues of the established *Plasmodium falciparum* 1-deoxy-D-xylulose-5-phosphate reductase (*Pf*DXR) enzyme inhibitor, FR900098. Bioassays of the targeted compounds and their synthetic precursors have revealed minimal antimalarial activity but encouraging anti-trypanosomal activity – in one case with an IC_{50} value of 5.4 µM against *Trypanosoma brucei*, the parasite responsible for Nagana (African cattle sleeping sickness). The results of relevant *in silico* modelling and docking studies undertaken in the design and evaluation of these compounds are discussed.

Keywords: Anti-parasitic, Anti-malarial, Anti-trypanosomal, *N*-benzylated phosphoramidate, *Nagana Trypanosoma brucei*



	R¹	R²
a	H	H
b	CH_2OH	H
c	NH_2	H
d	SH	H
e	Cl	Cl
f	Cl	H
g	F	F
h	F	H

*Corresponding author: Prof Perry Kaye. E-mail: p.kaye@ru.ac.za
†Present address: School of Biomedical Sciences, The University of Queensland, St. Lucia 4072 QLD, Brisbane, Australia.
‡ Present address: Department of Natural and Applied Sciences, Namibia University of Science and Technology, Namibia.

1. Introduction

While still unacceptably high, World Health Organization estimates of mortality levels arising from malarial infection[1] appear to have been declining in recent years. However, the emergence of resistance to even the newest antimalarial drugs[2] requires ongoing commitment to the development of effective replacements. Our earlier research[3-6] in this area has focussed on the synthesis and evaluation of novel carboxamido alkylphosphonic acid derivatives and *N*-substituted phosphoramidate esters[7] (as "reverse" fosmidomycin analogues) designed to act as inhibitors of *Plasmodium falciparum* 1-deoxy-D-xylulose-5-phosphate reductase (*Pf*DXR). This critical enzyme in the non-mevalonate isoprenoid biosynthetic pathway is unique to the mosquito vector but absent in the human host, and has been validated as an antimalarial drug target.[8,9] FR900098 **1**[10] is the *N*-acetyl derivative of the naturally occurring antibiotic, fosmidomycin **2** (Figure 1);[11] both compounds mimic the *Pf*DXR substrate and are effective *Pf*DXR inhibitors, but rapid *in vivo* clearance and recrudescence issues limit their usefulness.[12,13]

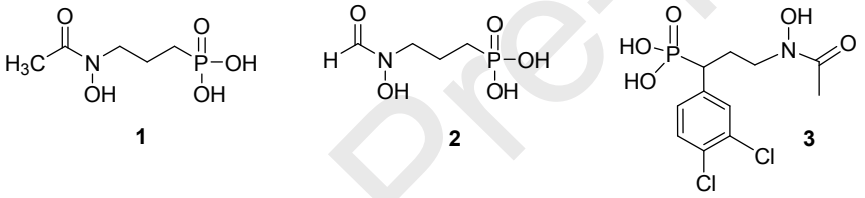


Figure 1. Structures of known *Pf*DXR inhibitors.

Nagana (African cattle sleeping sickness), another parasitic disease that has plagued the African continent, is a *Trypanosoma brucei* (*T.brucei*) infection due to subspecies, such as *T. brucei brucei*, carried by Tsetsi flies (*Glossina*). While control of this disease, which has been responsible for major losses in animal live-stock,[14] has improved dramatically, the available drugs are limited, old and susceptible to resistance.[15] Animals can also carry the subspecies, *Trypanosoma brucei gambiense*, responsible for most cases of human African trypanosomiasis (HAT).[16] Although new enzyme targets are being investigated,[17] to our knowledge, DXR has yet to be identified as a target for trypanosomal therapeutic intervention.

Preliminary analysis[18] of the 3-D topology of the active-site in a *homology-modelled* structure[19] of *Pf*DXR revealed the presence of three, adjacent, hydrophobic binding pockets. Perruchon[20] and Deng[21] had located two of these pockets close to the phosphonate moiety binding pocket, and the remarkable *Pf*DXR inhibition activity of the alkylphosphonate derivative **3** has been attributed[22] to the occupation of one of the empty pockets by the 3,4-dichlorophenyl group. Enantioselectivity

issues implicit in such chiral alkylphosphonates have prompted us to explore the preparation of non-chiral *N*-substituted phosphoramidate analogues[7] and, in this paper, we now report: i) the design and synthesis of a series of *N*-benzylated phosphoramidic acid analogues of FR900098 **1**; ii) their *in vitro* cytotoxicity and antimalarial and anti-trypanosomal activity; and iii) the results of *in silico* studies of their docking in the *Pf*DXR active site.

2. Results and Discussion

The novel phosphoramidate derivatives (**4**) were designed to incorporate the structural features illustrated in Figure **2**, *viz*., i) a phosphoramidate moiety in which the prochiral α-methylene group in the phosphonate ligands FR900098 **1** and fosmidomycin **2** is replaced by trigonal nitrogen, thus obviating the chirality issue; ii) an *N*-benzyl group to occupy a hydrophobic pocket adjacent to the *Pf*DXR active-site; iii) a three-atom aminoethyl (N-C-C) chain replacing the trimethylene hydrophobic patch in FR900098 **1**; and iv) the *N*-acetyl hydroxamate moiety present in FR900098 **1**, which is approximately twice as active *in vitro* as fosmidomycin **2**. It was recognised that the phosphoramidate esters (**4**) might serve as ester pro-drugs, exhibiting better lipophilicity than the phosphoramidic acids to which they could be hydrolysed *in vivo* by non-specific esterases.

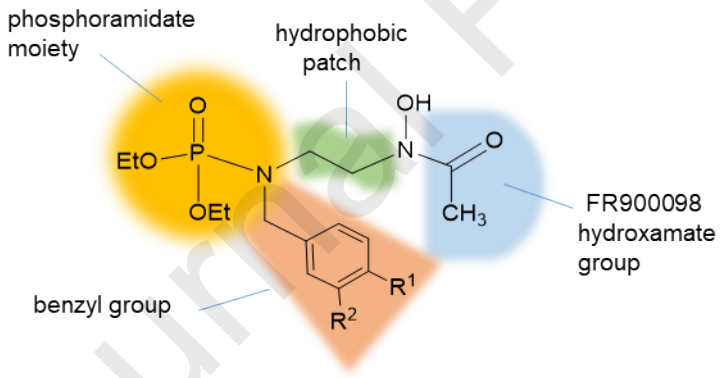


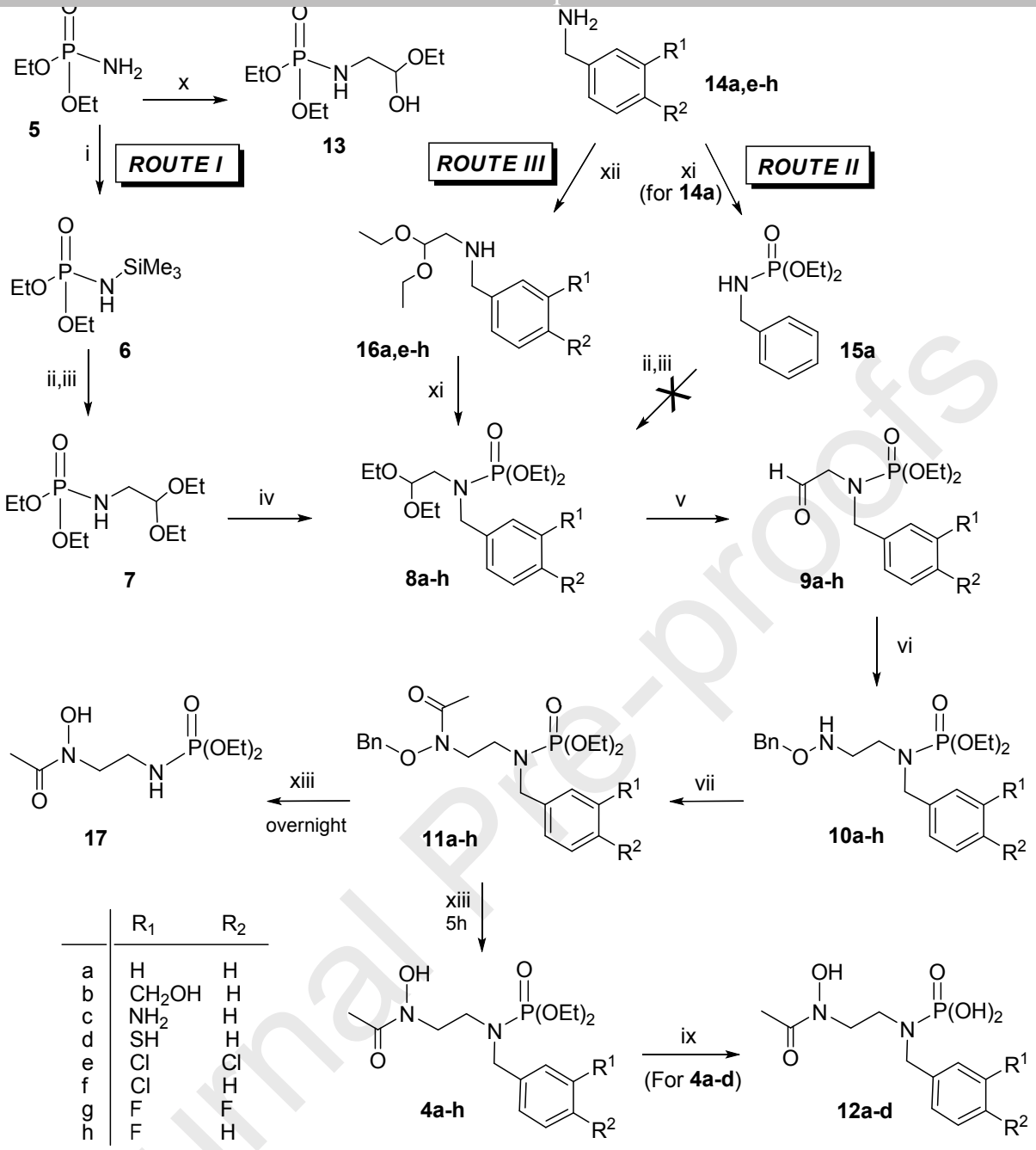
Figure 2. Design features of novel series of phosphoramidate ligands **4**.

The synthetic pathway (***Route I***) outlined in Scheme 1, adapted from reported methods for the preparation of fosmidomycin **2** and its analogues, was used to access the phosphoramidate esters **4a-d** and the corresponding phosphoramidic acids **12a-d**. Thus, using a method reported by Zwierzak *et al.*,[23] diethyl phosphoramidate **5** was converted to the silylated derivative **6** in essentially quantitative yield (98%). Acetalisation and benzylation steps afforded the *N*-benzylated phosphoramidate acetals **8a-d**, acid-catalysed hydrolysis of which afforded the corresponding aldehydes **9a-d** in excellent yields. Reductive amination of the aldehydes **9a-d**

using *O*-benzylhydroxylamine furnished the *O*-benzyl-protected amines **10a-d**. Incorporation of the acetyl functionality, required in the FR900098 analogues, was accomplished by adapting methodology reported by Ortmann *et al*.[24,25] to give the *O*-benzyl-protected *N*-acetyl derivatives **11a-d**. The final steps in the overall synthesis involved selective catalytic hydrogenolysis, using 10% Pd/C in methanol, to yield the hydroxamate ester derivatives **4a-h,** and hydrolysis of the esters, using TMSBr, to afford the novel FR900098 analogues **12a-d**.

A Saturation Transfer Difference (STD) NMR experiment[26] confirmed binding of the *N*-benzylated phosphoramidic acids **12a** and **12d** to the then available *Ec*DXR enzyme; the acids were used since the ester precursors were expected to undergo hydrolysis *in vivo* before binding to the enzyme. Preliminary *in silico* docking of the *N*-benzylated phosphoramidic acids **12a-d** was undertaken using then available, *homology-modelled* structure of *Pf*DXR.[19, 26] The ligands were observed to adopt binding orientations similar to that of fosmidomycin **2**, with the *N*-benzyl moieties occupying a hydrophobic pocket adjacent to the phosphonate-binding site. In each of the ligands **12a-d**, the phosphonate moiety exhibited hydrogen-bonding interactions with the active-site residues, Ser 199 and Gly 201, while the carbonyl group interacted with residue Ser 235. Additional interactions were observed between individual ligands **12a-d** and several amino acid residues in the active-site.

In view of the activity of the halogenated phosphonate esters reported by Haemers *et al*.,[22] the preparation of the initial series of phosphoramidic esters **4a-d** was extended to include halogenated derivatives. In a slight modification of the approach to the critical intermediates (**8**) (***Route I***, Scheme 1), the reaction of diethyl phosphoramidate **5** with hexamethyldisilazane was conducted in THF instead of benzene. However, the hemiacetal **13** was isolated repeatedly and alternative approaches, which involved introduction of a benzylamine moiety in the first step, were explored. Reaction of benzylamine **14a** with diethyl chlorophosphate in the presence of triethylamine (***Route II***) afforded the phosphoramidate derivative **15a**, but attempts to react this compound with bromoacetaldehyde diethyl acetal, in the presence of sodium hydride failed to afford the desired intermediate **8a**.



	R_1	R_2
a	H	H
b	CH_2OH	H
c	NH_2	H
d	SH	H
e	Cl	Cl
f	Cl	H
g	F	F
h	F	H

Scheme 1.

Reagents and conditions:- i) Hexamethyldisilazane (20% mol excess), benzene, 80 °C, 3 h; ii) NaH, benzene, r.t., N_2, then bromoacetaldehyde diethyl acetal, TBAB (10% mol), benzene, 80 °C, 4 h iii) EtOH, reflux, 1 h.; iv) NaH, benzyl halide, THF, r.t., 24 h, N_2; v) 2-M HCl, r.t., 24 h; vi) *O*-benzylhydroxylamine.HCl in MeOH, reflux, 5 h, then $NaCNBH_3$, conc. HCl, r.t.; vii) acetyl chloride, DCM, Et_3N, 0 °C, 1 h, then r.t., 24 h, N_2; viii) H_2, Pd/C, dry MeOH; ix) TMSBr, DCM, N_2, 0 °C, 1 h, then H_2O, r.t., overnight; x) hexamethyldisilazane, dry THF, 80 °C, 3 h; xi) diethyl chlorophosphate, Et_3N, DCM, r.t., 24 h; xii) bromoacetaldehyde diethyl acetal, anhydr. K_2CO_3, dry CH_3CN, 24 h, 85 °C; xiii) 10% Pd/C, dry MeOH, H_2.

In ***Route III***, initial *N*-alkylation of the benzylamines **14a,e-h**, using bromoacetaldehyde diethylacetal in the presence of anhydrous K_2CO_3 in refluxing acetonitrile, furnished the acetals **16a,e-h** in variable yield (23-61%), but subsequent reaction with diethyl chlorophosphate, in the presence of triethylamine, gave the diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)phosphor-amidate derivatives **8a,e-h** in good to excellent yield (71-94%). From this point, the original methodology described for ***Route I*** was essentially followed to access the phosphoramidate esters **4a,e-h**. However, purification of the *O*-benzylhydroxyamino compounds **10a,e-h** was problematic due to decomposition during column chromatography, and the subsequent conversion to the acetylated derivatives **11a**,**e-h** was successfully accomplished without prior purification of the precursors **10a,e-h**. Selective de-protection of the *N*-benzylated hydroxylamine moiety in the esters **11a**,**e-h** was effected in excellent yield (89-97%) by catalytic hydrogenation using 10% palladium-on-carbon catalyst in dry MeOH for a limited period (5 h) at room temperature. Initial reactions, conducted overnight and using compounds **11a,e**, proved to be non-selective, resulting in cleavage of both the *O*- and *N*-benzyl rings and affording the common product **17**, which is an aza-analogue of the diethyl phosphonate ester of FR900098 **1**. Careful TLC monitoring of the hydrogenolysis finally permitted selective removal of the *O*-benzyl group alone, to furnish the desired *N*-benzylated esters **4a**,**e-h**. Hydrolysis of the esters **4a-d** using TMSBr afforded the corresponding FR900098 analogues **12a-d**.

The *N*-benzylated phosphoramidate esters **4a-e** and **17** and the corresponding sets of intermediates (**9**), (**10**) and (**11**) were examined for: i) toxicity against HeLa cells; ii) antimalarial activity against *P. falciparum* using the plasmodium lactate dehydrogenase (pLDH) assay – a general screening expected to permit hydrolysis of ester derivatives due to the presence of esterases in the *Pf* cells; iii) anti-trypanosomal activity against *T.brucei*. The results are summarised in Table 1, and it is apparent that all of the compounds examined were found to be essentially non-toxic against HeLa cells at a concentration of 20 μM. Some of the 3,4-dichlorobenzyl derivatives showed a measure of antimalarial activity at a concentration of 20 μM, as determined by the pLDH assay of malarial cell viability (**8e** 54%; **9e** 85%; **10e** 70%; and **11e** 67%). More disappointingly, none of the targeted esters **4a,e-h** exhibited any statistically significant antimalarial (pLDH) activity. (The earlier, positive STD NMR results with compounds **12a** and **12d** had been obtained using the then available *Ec*DXR enzyme.) Surprisingly, compound **17**, which has a very similar structure to FR900098 **1**, also showed no promising activity against *P. falciparum* parasites.

Table1. Results for the HeLa cell toxicity and pLDH and *T.brucei* assays.

Ar	Compound	HeLa cell% viability at 20 µM	PLDH % activity at 20 µM	*T. brucei* % viability at 20 µM
Phenyl	**8a**	109.7±4.3	106.2±0.5	97.1±0.4
3,4-Dichlorophenyl	**8e**	101.0±2.1	53.9±6.5	2.6±0.3
3-Chlorophenyl	**8f**	98.2±5.1	90.5±3.9	101.7±9.4
3,4-Difluorophenyl	**8g**	119.1±4.9	127.9±3.8	102.8±1.1
3-Fluorophenyl	**8h**	80.9±10.7	121.3±2.8	112.0±4.7
Phenyl	**9a**	113.9±0.3	106.8±6.7	102.9±4.4
3,4-Dichlorophenyl	**9e**	91.1±2.1	85.4±5.0	96.2±0.8
3-Chlorophenyl	**9f**	114.0±6.7	113.9±2.6	90.0±2.7
3,4-Difluorophenyl	**9g**	95.8±13.5	95.5±8.9	102.2±16.7
3-Fluorophenyl	**9h**	103.9±11.1	106.8±0.1	97.4±10.3
Phenyl	**10a**	114.0±9.9	81.6±7.1	60.7±10.1
3,4-Dichlorophenyl	**10e**	101.1±4.9	69.8±7.9	2.1±0.1
3-Chlorophenyl	**10f**	115.5±0.6	85.5±10.7	26.5±31.2
3,4-Difluorophenyl	**10g**	93.5±11.5	119.9±8.5	112.2±1.6
3-Fluorophenyl	**10h**	96.0±13.4	101.2±6.8	118.1±1.9
Phenyl	**11a**	101.0±4.1	115.8±4.3	97.2±1.0
3,4-Dichlorophenyl	**11e**	98.7±0.5	66.5±12.5	1.2±0.3
3-Chlorophenyl	**11f**	115.8±6.4	93.6±4.0	9.2±0.2
3,4-Difluorophenyl	**11g**	98.1±6.9	85.5±4.3	31.8±0.1
3-Fluorophenyl	**11h**	90.4±0.2	102.5±2.0	60.2±2.8
-	**17**	105.6±5.5	101.3±0.2	106.4±3.2
Phenyl	**4a**	91.3±8.1	114.4±9.0	110.6±3.8
3,4-Dichlorophenyl	**4e**	119.8±8.4	103.1±7.6	103.9±2.6
3-Chlorophenyl	**4f**	89.6±5.9	104.1±2.8	117.8±2.9
3,4-Difluorophenyl	**4g**	99.6±11.2	100.0±1.4	104.4±10.7
3-Fluorophenyl	**4h**	117.2±0.3	115.9±0.4	108.2±0.7
	Control	0[a]	0[b]	0[c]

Positive control standards: [a]Emetine, [b]Chloroquine and [c]Pentamine.

Interestingly, a number of the compounds showed some activity against *T.brucei* (< *ca.* 60% cell viability) at a concentration of 20 µM, with four of them exhibiting significant activity (1.2 - 9.2% viability); IC_{50} values (Figure 3) were obtained for these four compounds, the 3,4-dichlorophenyl derivatives **8e** (13.9 µM), **10e** (16.9 µM) and **11e** (5.4 µM) and the 3-chlorophenyl analogue **11f** (13.2 µM). It is also interesting to note that three of these compounds (**8e**, **10e** and **11e**) also exhibit the best, albeit modest, antimalarial activity as determined by pLDH viability. To our knowledge, *T.brucei* does not contain a DXR enzyme, and the observed anti-trypanosomal activity is attributed to action at an unknown target.

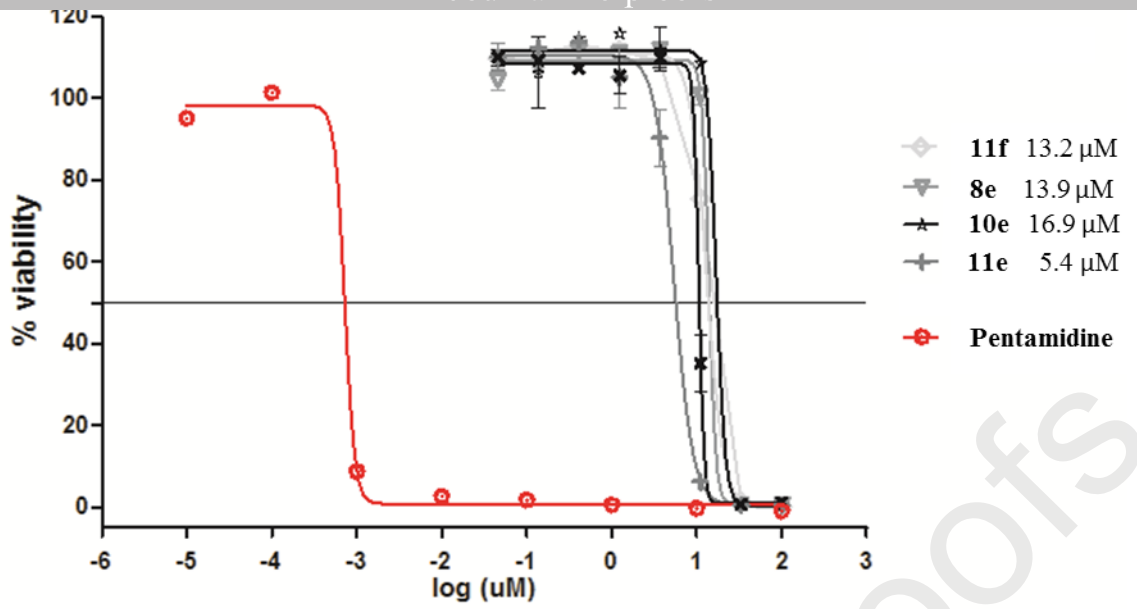
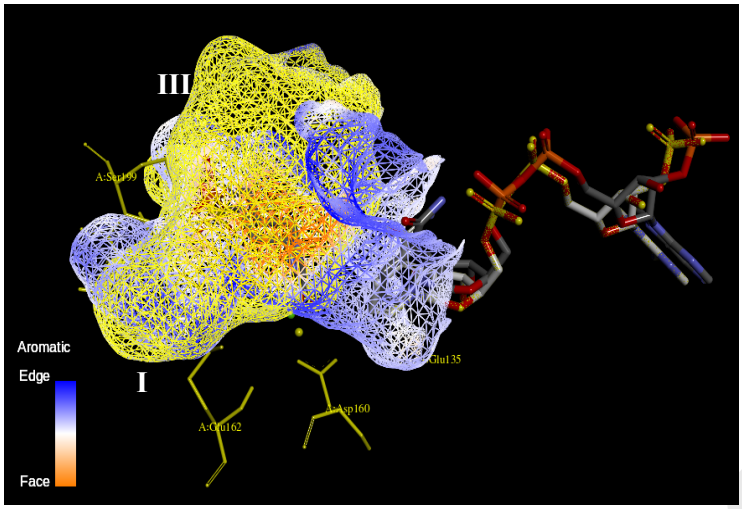


Figure 3. The inhibitory effects of selected compounds, showing IC_{50} values for *T.brucei* inhibition.

In order to rationalize the unexpectedly disappointing antimalarial data, *in silico* docking studies of the structures **12a-h** (and their synthetic precursors**)**, fosmidomycin **1** and FR900098 **2** were undertaken using the initially available, *homology-modelled Pf*DXR structure[19] *and* the *Pf*DXR structures (3AU9 and 3AUA)[9] which had subsequently been determined crystallographically. For the ligand models, both the negatively charged, mono-deprotonated phosphoramidic acid species and the phosphoramidate esters were constructed. Interestingly, many of the compounds, while docking within the active site of the *homology-modelled Pf*DXR[19] structure, rarely docked within the active sites of the *crystallographically determined Pf*DXR[9] structures. The failure of the targetted ligands to dock into the active-site of the *Pf*DXR structures is consistent with the observed lack of antimalarial activity.

In view of the evident discrepancies between the docking data obtained in the initial studies, using the then available *homology-modelled Pf*DXR[19] structure, and later docking studies[27] using the subsequently determined *X-ray crystallographic Pf*DXR structure,[9] attention has been given to exploring the relative topology of the respective active sites. Examination of the active sites of the *X-ray crystallographic Pf*DXR[9] and the *homology-modelled Pf*DXR[19] structures (Figures 4a,b) reveals that, while the overall volume of region I of the active site appears to remain comparable between the two structures, the overall volumes of regions II and III in the *X-ray crystallographic Pf*DXR[9] structure are considerably more compact. These differences presumably account for the failure of the targeted compounds to exhibit any significant anti-malarial activity.

(a)



(b)

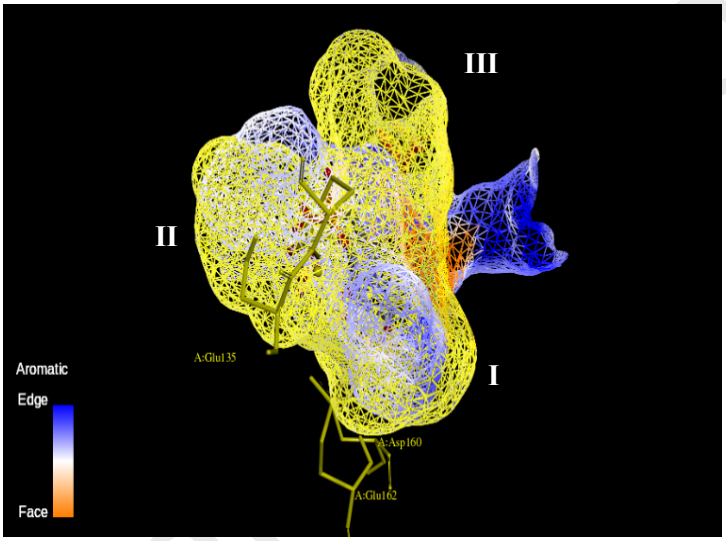


Figure 4. Different orientations (**a** and **b**) of the overlay of the of the *homology-modelled Pf*DXR active-site[19] (yellow) and the active-site (blue) in the *crystallographically determined Pf*DXR structure (3AU9).[9] In each case, the cofactor is present on the right and is coloured according to atom type.

3. Conclusions.

The *N*-benzylated phosphoramidate esters **4a-h** have been successfully synthesised as FR900098 ester analogues, using either a seven-step or a somewhat improved, six-step sequence. In a further step, selective hydrolysis of four of these esters has provided access to the corresponding phosphoramidic acids **12a-d**. While STD NMR experiments on two of these acids had supported their capacity to bind to the *Ec*DXR enzyme, the esters **4a,e-h** exhibited no statistically significant activity in pLDH assays. In contrast with the *in silico* docking data obtained using the *homology-modelled Pf*DXR enzyme structure,[19] the data obtained using the

crystallographically-determined PfDXR structure[7] indicated preferential binding of the acid analogues at a site slightly removed from the active site. These discrepancies are attributed to unexpected differences in the topologies of the active-sites. The observed activity of some of the synthetic intermediates against *T. brucei*, however, suggests their potential as lead compounds in the development of novel anti-trypanosomal agents.

4. Experimental

4.1. General methods

NMR spectra were typically recorded on Bruker 300, 400 or 600 MHz spectrometers in $CDCl_3$ and calibrated using solvent signals [δ_H: 7.26 ppm for residual $CHCl_3$. δ_C: 77.0 ppm ($CDCl_3$)]. Melting points were measured using a hot stage apparatus and are uncorrected. High-resolution mass spectra (HRMS) were recorded on a Waters API Q-TOF Ultima spectrometer (University of Stellenbosch, Stellenbosch, South Africa).

The known compound, diethyl *N*-(2,2-diethoxyethyl)phosphoramidate **7**[23] was isolated as a yellow oil (4.69 g, 87%). The benzyl halides, 3-(bromomethyl)aniline and 3-mercaptobenzyl bromide, were prepared, sequentially, from 3-aminobenzyl alcohol. General synthetic procedures and experimental data for representative compounds are reported below. Experimental data for the remaining synthesised compounds, NMR spectra for all new compounds, *in silico* docking protocols (and the resulting binding affinities) and the bioassay results are provided in the Supporting Information file. Bioassay protocols have been reported previously.[5]

4.2. The general procedure for the *N*-benzylation of the diethyl acetal 7 to access the intermediates 8a-d (*via Route I*) is illustrated by the following example. To a stirred solution of diethyl *N*-(2,2-diethoxyethyl)phosphoramidate **7** (1.00 g, 3.71mmol) in dry THF (20 mL) under N_2 was added NaH (60% dispersion in mineral oil; 0.20 g, 7.4 mmol) in small portions to permit controlled evolution of hydrogen. Benzyl bromide (0.44 mL, 3.7mmol) in dry THF (5 mL) was then added and the resulting solution was stirred for at room temperature for *ca.* 24 hours. The solvent was evaporated *in vacuo* and the residue extracted with EtOAc (2 x 25 mL). The organic phase was washed sequentially with satd. *aq*. $NaHCO_3$ (2 × 50 mL), water (2 x 50 mL) and brine (2 × 50 mL). The aqueous washings were extracted with EtOAc (2 x 25 mL) and the combined organic layers were dried (*anhydr*. $MgSO_4$). The solvent was removed *in vacuo* and the residue chromatographed [on silica gel; elution with hexane-EtOAc (4:6)] to yield *diethyl* N-*benzyl*-N-*(2,2-diethoxyethyl)phosphoramidate* **8a** as a yellow oil (0.60 g, 71%); (Found: C, 56.93; H, 8.49; N, 3.84%. $C_{17}H_{30}NO_5P$ requires C, 56.81; H, 8.41; N, 3.90%); ν_{max}/cm^{-1} 1232 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.20 (6H, t, J = 7.0 Hz, 2 × CH_3), 1.31 [6H, t, J = 7.1 Hz, $PO(OCH_2CH_3)_2$], 3.00 (2H, dd, $J_{H\text{-}H}$ = 5.4 and $J_{P\text{-}H}$ = 11.2 Hz, NCH_2), 3.48 (2H, m, 2 × OCH_aCH_3), 3.67 (2H, m, 2 × OCH_bCH_3), 4.07 [4H, m, $PO(OCH_2CH_3)_2$], 4.33 (2H, d, $J_{P\text{-}H}$ = 10.5 Hz, NCH_2Ph), 4.59 (1H, t, J = 5.4 Hz, CH), 7.25 (1H, d, J = 7.4 Hz, Ar-H), 7.31 (2H, t, J = 7.5 Hz, Ar-H) and 7.35 (2H, d, J = 7.5 Hz, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.5 (2 × CH_3), 16.3 [d, $J_{P\text{-}C}$ = 7.4 Hz, $PO(OCH_2CH_3)_2$], 47.4 (d, $J_{P\text{-}C}$ = 4.6 Hz, NCH_2), 50.6 (d, $J_{P\text{-}C}$ = 4.6 Hz, NCH_2Ph), 62.4 [d, $J_{P\text{-}C}$ = 5.6 Hz, $PO(OCH_2CH_3)_2$], 62.9 (2 × OCH_2CH_3), 102.8 (CH), 127.4, 128.5, 128.8 and 138.2 (Ar-C).

4.3. The general procedure for the *N*-phosphonation of the diethyl acetals (16) to access the intermediates 8a,e-h (*via Route III*) is illustrated by the following example. *N*-benzyl-2,2-diethoxyethanamine **16a** (3.79 g, 17.0 mmol) was dissolved DCM 20 mL, triethylamine (2.37 mL, 17.0 mmol) and diethyl chlorophosphate (2.45 mL, 17.0 mmol) was added to the reaction mixture and allowed to stir at r.t for 24 h. The mixture was washed with 10% HCl and then with water (2 × 20 mL). The organic layer was dried with *anhydr*. $MgSO_4$, filtered, and solvent was removed *in vacuo* to afford *diethyl* N-*benzyl*-N-*(2,2-diethoxyethyl)phosphoramidate* **8a** as a yellow oil (5.3 g, 87%).

4.4. The general procedure for the hydrolysis of the diethyl acetals (8) to afford the diethyl *N*-benzyl-*N*-(2-oxoethyl)phosphoramidates 9a-h is illustrated by the following example. Diethyl *N*-benzyl-*N*-(2,2-diethoxyethyl)phosphoramidate **8a** (5.2 g, 14 mmol) was dissolved in 45 mL of 2M HCl and stirred at rt for 24 h. The resulting mixture was dissolved in $CHCl_3$ and washed with water (3 × 20 mL), the water layer was re-extracted with $CHCl_3$and the combined organic layer was washed with $NaHCO_3$ (3 × 20 mL), brine (3 × 20 mL), dried with *anhydr.* $MgSO_4$ and concentration *in vacuo* afforded *diethyl* N-*benzyl*-N-*(2-oxoethyl)phosphoramidate* **9a** as a yellow oil (1.9 g, 45%) [HRMS: *m/z* calculated for $C_{13}H_{21}NO_4P$ (MH^+) 286.1208. Found. 286.1218]; ν_{max}/cm^{-1} 1695 (C=O) and 1230 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.37 [6H, t, J = 7.0 Hz, $PO(OCH_2CH_3)_2$], 3.77 (2H, d, $J_{P\text{-}H}$ = 11.6 Hz, NCH_2), 4.16 [4H, m, $PO(OCH_2CH_3)_2$], 4.28 (2H, d, $J_{P\text{-}H}$= 8.0 Hz, NCH_2Ph), 7.29-7.35 (5H, overlapping m, Ar-H) and 9.46 (1H, s, CHO); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, $J_{P\text{-}C}$ = 7.2 Hz, $PO(OCH_2CH_3)_2$], 51.1 (d, $J_{P\text{-}C}$ = 3.8 Hz, NCH_2), 55.4 (d, $J_{P\text{-}C}$ = 4.7 Hz, NCH_2Ph), 63.0 [d, $J_{P\text{-}C}$ = 5.5 Hz, $PO(OCH_2CH_3)_2$], 128.1, 128.8, 128.9 and 136.7 (Ar-C) and 199.9 (CHO).

4.5. The general procedure for reductive amination of the aldehydes (9) to afford the *N*-benzyloxy derivatives 10a-h is illustrated by the following example. To a stirred solution of diethyl *N*-benzyl-*N*-(2-oxoethyl)phosphoramidate **9a** (1.8 g, 6.48 mmol), in MeOH was added *O*-benzylhydroxylamine hydrochloride (1.35 g, 8.42 mmol). The solution was refluxed for 5 h and sodium cyanoborohydride (1.22 g, 19.4 mmol), conc. HCl (1.7 mL) was added dropwise and the mixture was stirred at r.t. for 1.5 h. Sodium cyanoborohydride (0.41 g, 0.48 mmol) was again added and the reaction mixture was stirred for 1h. The solvent was concentrated *in vacuo*, the residue was dissolved in MeOH (30 mL) and treated with ice water (20 mL) and the pH adjusted to 10 using aq. KOH solution. The solution was extracted using DCM (3 × 25 mL) and the combined organic layers were washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL) and dried with *anhydr*. $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (7:3)] to yield *diethyl* N-*benzyl*-N-*[2-(benzyloxyamino)ethyl]phosphoramidate* **10a** as a yellow oil (0.54 g, 21%) [HRMS: *m/z* calculated for $C_{20}H_{30}N_2O_4P$ (MH^+) 393.1943. Found 393.1946]; □$_{max}$/cm^{-1} 3408 (NH) and 1237 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 [6H, m, $PO(OCH_2CH_3)_2$], 3.06 (4H, m, NCH_2 and CH_2NH), 4.05 [4H, m, $PO(OCH_2CH_3)_2$], 4.10 (2H, d, $J_{P\text{-}H}$= 9.7 Hz, NCH_2Ph), 4.63 (2H, s, OCH_2Ph) and 7.26-7.31 (10H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, $J_{P\text{-}C}$ = 7.1 Hz, $PO(OCH_2CH_3)_2$], 43.0 (d, $J_{P\text{-}C}$ = 4.0 Hz, NCH_2), 50.2 (d, $J_{P\text{-}C}$ = 5.0 Hz, CH_2NH), 53.9 (NCH_2Ph), 62.6 [d, $J_{P\text{-}C}$ = 5.7 Hz, $PO(CH_2CH_3)_2$], 76.2 (OCH_2Ph), 127.5, 127.9, 128.3, 128.4, 128.5, 128.6, 138.0 and 138.1 (Ar-C).

4.6. The general procedure for the acetylation of the *N*-benzyloxy derivatives (10) to afford the amine derivatives 11a-h is illustrated by the following example. Acetyl chloride (0.24 mL, 3.4 mmol) was added dropwise into the chilled solution of diethyl *N*-benzyl-*N*-[2-(benzyloxyamino)ethyl]phosphoramidate **10a** (0.66 g, 1.7 mmol) and triethylamine (0.36 mL,

2.5 mmol) in dry DCM (10 mL). The reaction mixture was allowed to stir at 0 °C for 1 h, and then at r.t for 24 h. The solvent was removed *in vacuo* and the residue was dissolved in diethyl ether (15 mL). The solution was washed sequentially with *aq*. K_2CO_3 solution, 0.5M HCl and water. The organic solution was dried with *anhydr* $MgSO_4$, the solvent was concentrated to yield *diethyl* N-*benzyl*-N-*{2-[*N-*(benzyloxy)acetamido]ethyl}-phosphoramidate* **11a** as a yellow oil (0.23 g, 83%) [HRMS: *m/z* calculated for $C_{22}H_{32}N_2O_5P$ (MH^+) 435.2049. Found. 435.2046] (Found: C, 60.88; H, 7.23; N, 6.52%. $C_{22}H_{31}N_2O_5P$ requires C, 60.82; H, 7.19; N, 6.45%); ν_{max}/cm^{-1} 1683 (C=O) and 1246 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.30 [6H, m, $PO(OCH_2CH_3)_2$], 2.04 (3H, s, CH_3CO), 3.14 (2H, m, NCH_2), 3.71 (2H, m, CH_2NHCO), 4.04 [4H, m, $PO(OCH_2CH_3)_2$], 4.23 (2H, d, J_{P-H} = 9.1 Hz, NCH_2Ph), 4.75 (2H, s, OCH_2Ph) and 7.27-7.36 (10H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.3 [d, J_{P-C} = 7.2 Hz, $PO(OCH_2CH_3)_2$], 20.5 (CH_3CO), 42.0 (NCH_2), 43.6 (CH_2NCO), 50.0 (NCH_2Ph), 62.6 [d, J_{P-C} = 5.7 Hz, $PO(OCH_2CH_3)_2$], 76.4 (OCH_2Ph), 127.6, 128.6, 128.7, 128.8, 129.1, 129.3, 134.4 and 137.9 (Ar-C) and 172.4 (C=O).

4.7. The general procedure for the deprotection of the acetylated *N*-benzyloxy derivatives (11) to afford the phosphoramidate esters (4a-h) is illustrated by the following example.
A solution of diethyl *N*-benzyl-{2-[*N*-benzyloxy)acetamido]ethyl}phosphoramidate **11a** (0.12 g, 0.28 mmol) in dry MeOH (1 mL) was added to a solution of Pd/C (10%, 0.28 g) in dry MeOH (2 mL) saturated with hydrogen and the mixture was stirred at r.t. for 5 h. The reaction mixture was filtered through a celite pad and the filtrate was evaporated to yield *diethyl* N-*benzyl*-N-*{2-(*N-*hydroxyacetamido)ethyl}phosphoramidate* **4a** as a yellow oil (0.090 g, 91%) [HRMS: *m/z* calculated for $C_{15}H_{26}N_2O_5P$ (MH^+) 345.1579. Found. 345.1584]; ν_{max}/cm^{-1} 3160 (OH) and 1634 (C=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.34 [6H, t, *J* = 7.1 Hz, $PO(OCH_2CH_3)_2$], 2.18 (3H, s, CH_3CO), 3.19 (2H, m, NCH_2), 3.68 (2H, m, CH_2NCO), 4.05 [4H, m, $PO(OCH_2CH_3)_2$], 4.12 (2H, d, J_{P-H}= 9.3 Hz, NCH_2Ph), 7.31 (3H, m, Ar-H) and 7.35 (2H, m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.3 Hz, $PO(OCH_2CH_3)_2$], 20.9 (CH_3CO), 41.0 (d, J_{P-C} = 5.2 Hz, NCH_2), 43.3 (CH_2NCO), 49.1 (d, J_{P-C} = 3.6 Hz, NCH_2Ph), 63.3 [d, J_{P-C} = 6.1 Hz, $PO(OCH_2CH_3)_2$], 127.9, 128.5, 128.8 and 136.8 (Ar-C) and 172.9 (C=O).

4.8. General procedure for hydrolysis of the phosphoramidate esters (4a-d) to afford the phosphoramidic acids 12a-d is illustrated by the following example. Trimethylsilyl bromide (0.15 mL, 1.0 mmol) was added dropwise to diethyl *N*-benzyl-2-(*N*-hydroxyacetamido)-ethylphosphoramidate **4a** (0.12 g, 0.34 mmol) in DCM (5 mL) under N_2 at 0 °C and the mixture was stirred for 1 hour. The mixture was allowed to warm to room temperature, water was added (1 mL) and the resulting mixture was stirred overnight. After completion, the solvent was removed *in vacuo* and the residue chromatographed [preparative layer chromatography; elution with hexane-EtOAc-MeOH (1:1:1)] to yield N-*benzyl-2-(*N-*hydroxyacetamido)-ethylphosphoramidic acid* **12a** as a colourless oil (85 mg, 87%); (Found: C, 45.76; H, 6.01; N, 9.79%. $C_{11}H_{17}N_2O_5P$ requires C, 45.84; H, 5.94; N, 9.72%); ν_{max}/cm^{-1} 3245 (OH), 1653 (C=O) and 1228 (P=O); δ_H/ppm (400 MHz; DMSO-d_6) 2.09 (3H, s, CH_3CO), 2.79 (2H, m, NCH_2), 3.34 (2H, t, *J* = 6.4 Hz, CH_2NCO), 3.83 (2H, s, NCH_2Ph), 5.25 (1H, s, NOH), 7.29 - 7.37 (5H, m, Ar-H) and 8.15 (2H, s, 2 x OH); δ_C/ppm (100 MHz; DMSO-d_6) 20.7 (CH_3CO), 42.6 (d, J_{P-C} = 4.7 Hz, NCH_2), 52.3 (d, J_{P-C} = 16.9 Hz, CH_2NCO), 67.2 (NCH_2Ph), 126.4, 128.6, 129.4 and 139.4 (Ar-C) and 171.5 (C=O).

4.9. The general procedure for the preparation of the *N*-benzyl-2,2-diethoxyethanamine derivatives 16a,e-h is illustrated by the following example. Bromoacetaldehyde diethyl acetal (4.2 mL, 28 mmol) was added to a solution of benzylamine **9a** (3.0 mL, 28 mmol) and anhydrous K_2CO_3 (3.9 g, 28 mmol) in dry acetonitrile (15 mL) and the mixture was refluxed for 24 h. The

mixture was diluted with EtOAc (20 mL) and washed sequentially with water (3 × 25 mL) and dried with *anhydr* $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography [on silica gel; elution with EtOAc-hexane (6:4)] to afford *N*-benzyl-2,2-diethoxyethanamine **16a**[30] as a yellow oil (3.8 g, 61%); ν_{max}/cm^{-1} 3399 (NH); δ_H/ppm (600 MHz; CDCl3) 1.20 (6H, t, J = 7.1 Hz, 2 × CH_3), 2.75 (2H, d, J = 5.6 Hz, NCH_2), 3.53 (2H, m, 2 × OCH_aCH_3), 3.68 (2H, m, 2 × OCH_bCH_3), 3.81 (2H, s, NCH_2Ph), 4.62 (1H, t, J = 5.6 Hz, CH), 7.25 (1H, m, Ar-H) and 7.32-7.33 (4H, overlapping m, Ar-H); δ_C/ppm (150 MHz; $CDCl_3$) 15.6 (2 × CH_3), 51.7 (NCH_2), 54.0 (NCH_2Ph), 62.5 (2 × OCH_2CH_3), 102.3 (CH), 127.1, 128.3, 128.5 and 140.3 (Ar-C).

4.10. Diethyl *N*-{2-(*N*-hydroxyacetamido)ethyl}phosphoramidate 17

A solution of diethyl *N*-benzyl-{2-[*N*-benzyloxy)acetamido]ethyl}phosphoramidate **14a** (0.12 g, 0.28 mmol) in dry MeOH (1 mL) was added to a solution of Pd/C (10%, 0.28 g) in dry MeOH (2 mL) saturated with hydrogen and the mixture was stirred overnight at r.t. The reaction mixture was filtered through celite pad and the filtrate was evaporated to yield *diethyl* N-*{2-(*N-*hydroxyacetamido)ethyl}phosphoramidate* **17** as a pale-yellow oil (0.063 g, 90%) [HRMS: *m/z* calculated for $C_8H_{20}N_2O_5P$ (MH^+) 255.1110. Found. 255.1103]; ν_{max}/cm^{-1} 3222 (OH), 1623 (C=O) and 1209 (P=O); δ_H/ppm (600 MHz; $CDCl_3$) 1.28 [6H, m, $PO(OCH_2CH_3)_2$], 2.15 (3H, s, CH_3CO), 3.18 (2H, m, NCH_2), 3.67 (2H, m, CH_2NCO), 3.84 (1H, s, NH), 3.99 [4H, m, $PO(OCH_2CH_3)_2$] and 9.70 (1H, s, OH); δ_C/ppm (150 MHz; $CDCl_3$) 16.2 [d, J_{P-C} = 7.1 Hz, $PO(OCH_2CH_3)_2$], 20.7 (CH_3CO), 37.9 (NCH_2), 48.3 (CH_2NCO), 63.1 [d, J_{P-C} = 5.7 Hz, $PO(OCH_2CH_3)_2$] and 173.2 (C=O).

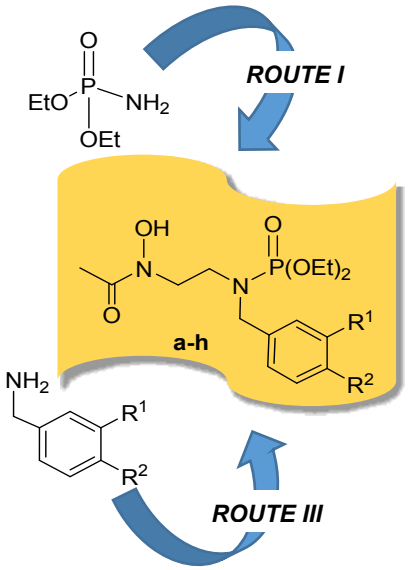
Acknowledgements

The authors thank Rhodes University and the South African Medical Research Council (SAMRC) for generous financial support and the SAMRC for a bursary (to C.M.A.). This research project was funded by the South African Medical Research Council (SAMRC) with funds from National Treasury under its Economic Competitiveness and Support Package. A.C.C. was supported by a Beit Trust scholarship.

References

1. World Health Organization Report, **2012**. *https://ourworldindata.org/malaria/* (22/03/2017).
2. Hyde, J.E. *Acta Trop*., **2005**, *94*, 191-206.
3. Bodill, T., Conibear, A.C., Blatch, G.L., Kevin A. Lobb, K.A. and Kaye, P.T. *Bioorg. & Med. Chem*., **2011**, *19*, 1321-1327.
4. Bodill, T., Conibear, A.C., Marius K. M. Mutorwa, M.K., Goble, J.L., Blatch, G.L., Lobb, K.A., Klein, R. and Perry T. Kaye, P.T., *Bioorg. Med. Chem*., **2013**, *21*, 4332- 4341.
5. Adeyemi, C.M., Faridoon, Isaacs, M., Mnkandhla, D., Hoppe, H.C., Krause, R.W.M. and Kaye, P.T. *Bioorg. & Med. Chem.*, **2016**, *24*, 6131-6138.
6. Adeyemi, C.M., Klein, R., Isaacs, M., Mnkandhla, D., Hoppe, H.C., Krause, R.W.M. and Kaye, P.T. *Tetrahedron,* **2017**, *73*, 1661-1667.

7. Adeyemi, C.M., Hoppe, H.C., Isaacs, M., Lobb, K.A., Klein, R. and Kaye, P.T. *Tetrahedron*, **2019**, *75*(16), 2371-2378.
8. Singh, N., Cheve, G., Avery, M.A., and McCurdy, C.R. *Curr. Pharm. Des*., **2007**, *13*, 1161-1177.
9. Umeda, T., Tanaka, N., Kusakabe, Y., Nakanishi, M., Kitade, Y. and Nakamura, K.T. *Scientific Reports*, **2011**, *1*(9), 1-9.
10. Jomaa, H. Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Türbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H.K., Soldati, D. and Beck, E. *Science* **1999***, 285*(5433), 1573-1576.
11. Lichtenthaler, H.K. *Biochem. Soc. Trans.* **2000***, 28*(6), 785-789.
12. Murakawa, T., Sakamoto, H., Fukada, S., Konishi, T. and Nishida, M. *Antimicrob. Agents Chemother.,* **1982**, *21*(2), 224-230.
13. Lienaua,C., Konzuch, S., Gräwert, T., Illarionov, B., Bacher, A., Fischer, M., Tanaka, N. and Kurz, T. *Procedia Chemistry* **2015**, *14*, 108-116
14. Meyer, A., Holt, H.R., Selby, R., Guitian, J., *PLoS Negl. Trop. Dis*., **2016**, *10*(12): e0005247.
15. Yaro, M., .Munyard, K.A., Stear, M.J. and .Groth, D.M. *Vet. Parasitol.*, **2016**, *225,* 43-52.
16. Trypanosoma brucei gambiense What is African sleeping sickness? | Facts | yourgenome.org https://www.yourgenome.org/facts/what-is-african-sleeping-sickness (10/01/2018).
17. Scotti L., Mendonça, F.J.B., da Silva, M.S. and Scotti, M.T. *Current Protein & Peptide Science*, **2016**, *17*(3), 243-249.
18. Conibear, A.C. *Synthesis and Evaluation of Novel Inhibitors of 1-Deoxy-D-xylulose-5-phosphate Reductoisomerase as Potential Antimalarials,* MSc. Thesis, Rhodes University, Grahamstown, **2010**. Figure provided in the Supporting Information.
19. Goble, J.L., Adendorff, M.R., de Beer, T.A.P., Stephens L.L. and Blatch G.L., *Protein Pept. Lett.* **2010**, *17*, 109-120.
20. Perruchon, J., Ortmann, R., Altenkamper, M., Silber, K., Wiesner, J., Jomaa, H., Klebe, G. and Schlitzer, M. *ChemMedChem*., **2008**, *3*, 1232-1241.
21. Deng, L., Sundriyal, S., Rubio, V., Shi, Z. and Song, Y., *J. Med. Chem*., **2009**, *52*, 6539- 6542.
22. Haemers, T., Wiesner, J., Van Poecke, S., Goeman, J., Henschker, D., Beck, E., Jomaab, H. and Van Calenbergha, S., *Bioorg. Med. Chem. Lett.*, **2006**, *16*(7), 1888–1891.
23. Zwierzak A., *Synthesis,* **1982**, *11*, 920-922.
24. Ortmann, R., Wiesner, J., Reichenberg, A., Henschker, D., Beck, E., Jomaa, H. and Schlitzer, M., *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 2163-2166.
25. Ortmann, R., Wiesner, J., Reichenberg, A., Henschker, D., Beck, E., Jomaa, H. and Schlitzer, M. *Arch. Pharm. Chem. Life Sci.*, **2005**, *338*, 305−314.
26. Mutorwa, M.K., Synthesis of Novel Inhibitors of 1-Deoxy-D-xylulose-5-phosphate Reductoisomerase as Potential Anti-malarial Lead Compounds, Ph.D. Thesis, Rhodes University, Grahamstown, **2011**.
27. Adeyemi, C.M. Synthesis and Biological Screening of Potential Plasmodium Falciparum DXR Inhibitors. Ph.D. Thesis, Rhodes University, Grahamstown, **2016**.



	R¹	R²
a	H	H
b	CH_2OH	H
c	NH_2	H
d	SH	H
e	Cl	Cl
f	Cl	H
g	F	F
h	F	H

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

1. Design and successful development of synthetic pathways to novel phosphoramidic acid derivatives.
2. Bioassay data reveals the anti-trypanosomal potential of some of the compounds.
3. Detailed in silico modelling and docking studies have been undertaken to explore the topology of the actives site in an initial homology-modelled *Pf*DXR enzyme strycture and in a subsequent, crystallographically determined *Pf*DXR structure, the results of which account for the unexpected lack of antimalarial activity.