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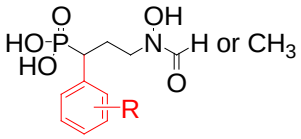

A series of α-substituted fosmidomycin analogues was synthesized and evaluated for DOXP reductoisomerase inhibition and *P. falciparum* growth inhibition. In the latter assay most analogues proved superior to fosmidomycin.

Synthesis of α-substituted fosmidomycin analogues as highly potent *P. falciparum* growth inhibitors

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Abstract

In view of the promising antimalarial activity of fosmidomycin or its *N*-acetyl homologue FR900098, the objective of this work was to investigate the influence of aromatic substituents in the alpha position of the phosphonate moiety. The envisaged analogues were prepared using a linear route involving a 3-aryl-3-phosphoryl propanal intermediate. The activities of all compounds were evaluated on *E. coli* 1-deoxy-D-xylulose 5-phosphate reductoisomerase and against two *Plasmodium falciparum* strains. Compared with fosmidomycin, several analogues displayed enhanced activity towards the *P. falciparum* strains. Compound **1e** with a 3,4-dichlorophenyl substitution in the α-position of fosmidomycin emerged as the most potent analogue of this series. It is approximately three times more potent in inhibiting the growth of *P. falciparum* than FR900098, the most potent representative of this class reported so far.

Malaria is estimated to kill more than one million people annually and possibly as many as three million, with most of the deaths among children under age six living in sub-Saharan Africa. As a result of the resurgence of malaria and increased resistance to prevalent anti-malarials such as chloroquine, there is an urgent need for new efficient chemotherapeutics against this disease.[1] Drug development has shifted toward targeting specific proteins that are unique and critical for cellular growth and survival of the parasite.[2,3] Recently, the presence of a mevalonate-independent pathway for isoprenoid biosynthesis in *P. falciparum* was discovered.[4,5] 1-Deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase plays an essential role in this non-mevalonate pathway, which is absent in humans. Previous studies demonstrated that fosmidomycin exerts potent antimalarial activity by inhibition of DOXP reductoisomerase (DXR), the second enzyme in the reaction cascade.[6,7] In recent clinical trials conducted in Gabon and Thailand, fosmidomycin proved to be efficient in the treatment of patients suffering from acute, uncomplicated *P. falciparum* malaria.[8,9] Fosmidomycin has the advantage to be remarkably nontoxic and to exhibit activity against multiresistant parasite strains. Limitations are the short plasma half-life and the moderate resorption rate.[1] The acetyl derivative of fosmidomycin, FR900098, was shown to be approximately twice as active against *P. falciparum in vitro* as well as in a *P. vinckei* mouse model.

[Figure 1]

Fosmidomycin also represents a valuable lead for further modification. In order to study the structure-activity relationships, hydroxamic moiety modifications, including benzoxazolone and oxazolopyridinone functionaltities, have been reported.[10] Also, the phosphonate moiety has been altered to produce prodrugs with improved oral bioavailability.[11,12,13]

Interestingly, modifications addressing the three carbon spacer are scarce. The objective of this work was to synthesize a series of fosmidomycin or FR900098 analogues containing a phenyl moiety in the α-position. To sort out the influence of lipophilicity and electronic properties of this phenyl moiety, substituents were introduced according to Topliss' methodology.[14] Briefly, in this methodology an operational scheme is used to quickly identify the optimum substitution on a benzene ring for maximizing drug potency by virtue of resulting changes in hydrophobic, electronic and steric effects.

Retrosynthetic analysis toward the synthesis of the desired analogues is depicted in Scheme 1. 3-Aryl substituted 3-phosphoryl propanal was anticipated to be a convenient precursor.

[Scheme 1]

Two synthetic pathways toward this aldehyde synthon were explored (Scheme 2). The first route started from allyl bromide which upon Arbusow reaction with the appropriate diethyl benzylphosphonate in the presence of *n*-BuLi, afforded **4a**,**b** in 97 and 33 % yield.[15] Oxidation of **4a**,**b** to the vicinal *cis*-diol, followed by sodium periodate cleavage gave aldehydes **7a**,**b**.

[Scheme 2]

When the desired benzylphosphonate was not commercially available, an alternative strategy to prepare the desired aldehydes was followed.[16] A 1,4-addition of triethyl phosphite to the appropriately substituted cinnamaldehyde in the presence of phenol gave the acetals **6c-e** in 70-85 % yield. Subsequent deprotection afforded in 76-83 % yield the corresponding aldehydes, which were stable enough to be purified by flash chromatography. If necessary, substituted cinnamaldehydes were synthesized. Several procedures are described in the literature. In our hands a palladium-catalyzed synthesis from acrolein diethyl acetal and the corresponding aryl iodide was very efficient.[17]

[Scheme 3]

The remainder of the synthesis is depicted in Scheme 3. Treatment of **7a-e** with *O*-benzylhydroxylamine yielded (67-92 %) oximes **8a-e**, which were reduced with sodium cyanoborohydride to produce benzyloxyamines **9a-e** in 91-96 % yield. Subsequent acylation afforded **10c**,**d**,**e** and **11a-e** in good yield. Benzyl deprotection by catalytic hydrogenation gave **12c**,**e** and **13a-e**, which occurred as mixtures of two hydroxamic acid isomers (*syn* and *anti*). Generally, hydrogenolysis of the benzyl group was accompanied by partial deoxygenation, while attempted deprotection of compound **10d** using hydrogenolysis or BCl_3 led to loss of the formyl moiety. Compounds **12c**,**e** and **13a-e** were finally deprotected with TMSBr in CH_2Cl_2 to afford pure **1c**,**e** and **2a-e** after purification by reversed phase HPLC.[18]

All final compounds were tested for inhibition of recombinant *E. coli* DXR. The assay used was based on the photometric measurement of the NADPH turnover associated with the DXR catalysed reaction.[7] In addition, the in vitro antimalarial activity of the compounds was determined. Intraerythrocytic stages of the *P. falciparum* strains D2d or 3D7 were incubated with serial dilutions of the drugs and the viability of the parasites assessed by their ability to incorporate [^{3}H]hypoxanthine into DNA.[19] Fosmidomycin and FR900098 were included as reference compounds.

Compared with fosmidomycin, all investigated analogues were weaker inhibitors of *E. coli* DXR (Table 1). Analysis of the order of DXR inhibitory potency in the *N*-acetyl series **2a-e** (**2d** ≈ **2e** > **2a** > **2b** > **2c**) indicates that the activity is mainly +σ-controlled (4-Cl ≈ 3,4-diCl > H > 4-Me > 4-MeO). Although generally weaker than fosmidomycin in inhibiting *E. coli* DXR, all α-substituted analogues studied ~~However, several of them significantly~~ surpass~~ed~~ the activity of fosmidomycin to inhibit the parasite growth. Remarkably, the formyl analogues **1c** and **1e** consistently outperformed the acetyl derivatives **2c** and **2e**, both in the enzyme and the parasite growth inhibition assay. An opposite trend was observed for the fosmidomycin/FR900098 couple in the parasite growth inhibition assay, in accordance with previous studies.[4] Interestingly, a worthwhile correlation was observed between the IC_{50} values of **1c**, **1e** and **2a-e** for DXR and their IC_{50} for the malaria strains, indicating that *E. coli* DXR inhibition is a useful predictor to estimate the *in vitro* antimalarial activity. However, this correlation only seems to hold when considering a set of closely related analogues. Indeed, while fosmidomycin is clearly superior to the α-substituted analogues as DXR inhibitor, it is less active in inhibiting *P. falciparum* growth.

Compound **1e** emerged as the most promising analogue of this series. Its *in vitro* antimalarial activity indicated that it is twelve-fold more active than fosmidomycin and also exceeds the activity of FR900098, the most potent analogue known to date. Apparently, the lipophilic and electronegative properties of the 3,4-dichloro-substitution pattern selectively favour the interaction with the *P. falciparum* DXR homologue. Alternatively, the aromatic ring in the α-position may improve the capacity of the compounds to enter the parasite cells.

In summary, a practical method for the synthesis of α-aryl substituted fosmidomycin analogues was developed. Several analogues were superior to fosmidomycin in inhibiting the growth of malaria parasites.

AKNOWLEDGEMENT This study was supported by grants from the European Commission (QLK2-CT-2002-00887) and INTAS (03-51-4077).

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[18] All final compounds were purified using a preparative HPLC system on a C18 column (5µm; Phenomenex; Luna; 250 x 21.2 mm) with a linear gradient of acetonitrile in 5 mM NH_4OAc solution over 20 min at a flow rate of 17.5 mL/min. The purity of all target compounds was assessed by analytical HPLC (5µm; Phenomenex; C18(2); 250 x 4.6 mm) using the same gradient at a flow rate of 1 mL/min. Spectral data for analogue **1e**: ^{1}H NMR (300 MHz; D_20) δ = 2.07 (m, 1H, β-CH); 2.31 (m, 1H, β-CH); 2.80 (m, 1H, α-CH); 3.32 (m, 2H, γ-CH_2); 7.10 (m, 1H, arom. H); 7.36 (m, 2H, arom. H); 7.44 and 8.07 (2 x s, 1H, major and minor HC=O) ppm. ^{13}C NMR (75 MHz; D_2O) δ = 26.49 (s, β-CH_2) ; 42.82 (d, α-CH, $^{1}J_{PC}$ = 129.6 Hz); 48.86 (d, γ-CH_2, $^{3}J_{PC}$ = 17.0 Hz); 128.92 (d, J_{PC} = 5.8 Hz, =CH); 130.04 (d, J_{PC} = 3.8 Hz, =C) ; 130.55 (d, J_{PC} = 2.6 Hz, =CH); 130.73 (d, J_{PC} = 6.0 Hz, =CH); 131.88 (d, J_{PC} = 3.2 Hz, =C); 138.80 (d, J_{PC} = 7.2 Hz, =C); 159.70 and 163.76 (2 x s, major and minor C=O) ppm. ^{31}P NMR (121 MHz; D_2O) δ = 21.46 and 21.78 (major and minor isomer); mass (ESI-MS) 329.9 $[M+H]^+$.
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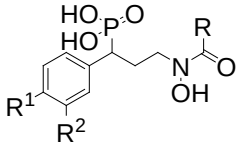


compound	R	R^1	R^2	IC_{50} (µM) *E. coli* DXR[1]	IC_{50} (µM) Dd2	IC_{50} (µM) 3D7
2a	CH_3	H	H	0.311 ± 0.120	0.35	0.55
2b	CH_3	Me	H	0.396 ± 0.061	0.22	0.95
1c	H	OMe	H	0.156 ± 0.043	0.20	0.36
2c	CH_3	OMe	H	0.459 ± 0.109	0.27	0.85
2d	CH_3	Cl	H	0.099 ± 0.026	0.095	0.35
1e	H	Cl	Cl	0.059 ± 0.020	0.028	0.090
2e	CH_3	Cl	Cl	0.119 ± 0.019	0.090	0.25
fosmidomycin				0.030 ± 0.008	0.36	1.1
FR900098				0.030 ± 0.008	0.18	0.32

[1]Mean values ± standard deviation of 3 to 5 independent measurements

Table 1. IC_{50} values against recombinant *E. coli* DXR and Dd2 and 3D7 *P. falciparum* strains

Legends

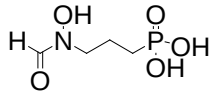
Figure 1. Structures of fosmidomycin, FR900098 and analogues under study.

Scheme 1. Retrosynthetic route toward analogues **1** and **2**.

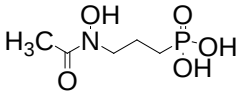
Scheme 2. Reagents and conditions: (a) (i) n-BuLi, THF, -50 to -70 °C, (ii) allyl bromide, -70 °C; (b) (i) OsO_4, 4-methyl-morpholine N-oxide, dioxane (ii) $NaIO_4$; (c) triethyl phosphite, phenol, 100 °C; (d) 2N HCl, rt.

Scheme 3. Reagents and conditions: (a) *O*-benzylhydroxylamine, pyridine, EtOH, rt; (b) $NaCNBH_3$, MeOH, HCl, rt; (c) Acetyl chloride, CH_2Cl_2, EtN_3, 0 °C or carbonyldiimidazole, HCOOH, CH_2Cl_2, rt; (d) H_2, Pd/C, MeOH, rt; (e) TMSBr, CH_2Cl_2, rt.

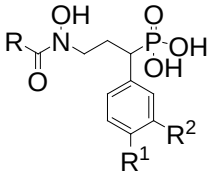
Figure 1



Fosmidomycin



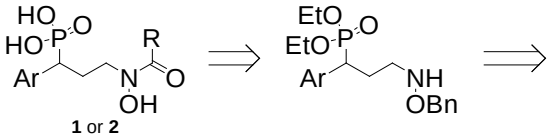
FR900098



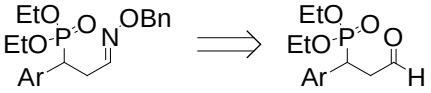
1: R = H
2: R = CH_3

a: $R^1 = R^2 = H$
b: R^1 = Me; R^2 = H
c: R^1 = OMe; R^2 = H
d: R^1 = Cl; R^2 = H
e: $R^1 = R^2$ = Cl

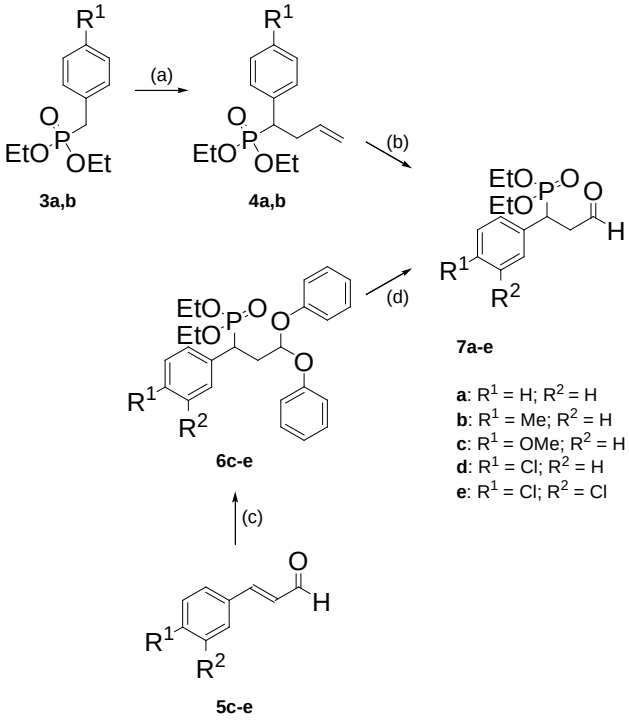
Scheme 1



1 or **2**



Scheme 2



3a,b

4a,b

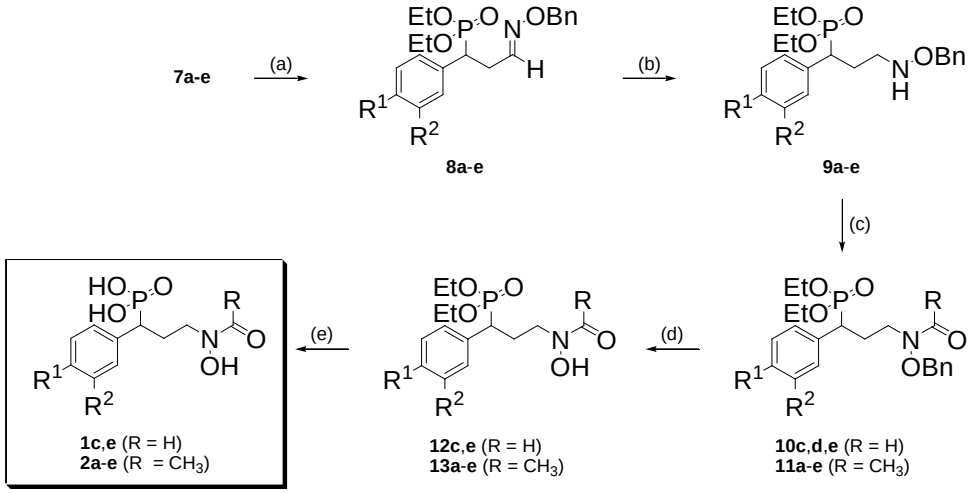
5c-e

6c-e

7a-e

a: $R^1 = R^2 = H$
b: R^1 = Me; R^2 = H
c: R^1 = OMe; R^2 = H
d: R^1 = Cl; R^2 = H
e: $R^1 = R^2$ = Cl

Scheme 3


7a-e
(a)
8a-e
(b)
9a-e
(c)
10c,d,e (R = H)
11a-e (R = CH3)
(d)
12c,e (R = H)
13a-e (R = CH3)
(e)
1c,e (R = H)
2a-e (R = CH3)