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Synthesis and evaluation of α,β-unsaturated α-aryl-substituted fosmidomycin analogues as DXR inhibitors

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Abstract—Fosmidomycin, which acts through inhibition of 1-deoxy-D-xylulose phosphate reductoisomerase (DXR) in the non-mevalonate pathway, represents a valuable recent addition to the armamentarium against uncomplicated malaria. In this paper, we describe the synthesis and biological evaluation of *E*- and *Z*-α,β-unsaturated α-aryl-substituted analogues of FR900098, a fosmidomycin congener, utilizing a Stille or a Suzuki coupling to introduce the aryl group. In contrast with our expectations based on the promising activity earlier observed for several α-substituted fosmidomycin analogues, all synthesized analogues exhibited much lower binding affinity for DXR than fosmidomycin.

With over 500 million clinical cases each year, malaria still remains a major threat in the world, as more than two million people succumb from the disease each year. The high prevalence of the disease is attributed to the difficulties in vector control, as well as the increasing resistance of *Plasmodium falciparum*, the main causative agent of the disease, towards the commonly used antimalarials such as chloroquine. Therefore, new antimalarial drugs acting on alternative, yet unexplored biochemical pathways are urgently needed.

Recently, the discovery of the mevalonate-independent pathway for isoprenoid biosynthesis opened the way for new therapeutics to cure malaria, as this alternative pathway is absent in humans. Two groups simultaneously discovered that fosmidomycin effectively inhibits 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR).[1,2] This essential enzyme converts 1-deoxy-D-xylulose 5-phosphate (DOXP) to 2*C*-methyl-D-erithrytol phosphate (MEP), the second step in the non-mevalonate pathway. In recent clinical trials fosmidomycin combinations with clindamycin[3] or artesunate[4] were found very efficient in curing *P. falciparum* uncomplicated malaria (Fig. 1). FR900098, the acetyl congener of fosmidomycin, was found to be twice as active as fosmidomycin, both in vitro and in a *Plasmodium vinckei* mouse model.[2]

For structure–activity relationship analyses of fosmidomycin derivatives, various alterations have been made, mainly addressing the retrohydroxamate and phosphonate moieties.[5–9] Comparatively few modifications of the carbon spacer have been explored. Hence, we recently focused on such type of modifications (Fig. 1).

Incorporation of aryl functionalities in α-position of the phosphonate of fosmidomycin (e.g., **1**) or FR900098 resulted in a markedly enhanced in vitro antiplasmodial activity, which proved to be associated to the electron withdrawing properties of the aryl substituents.[10,11]

Based on a reported prodrug approach,[6] Kurz et al. systematically investigated the effect of introduction of different substituents in α-position of the bis(pivaloyloxymethyl)esters of fosmidomycin or FR900098.[12–14] Introduction of an α-methyl or α-phenyl substituent

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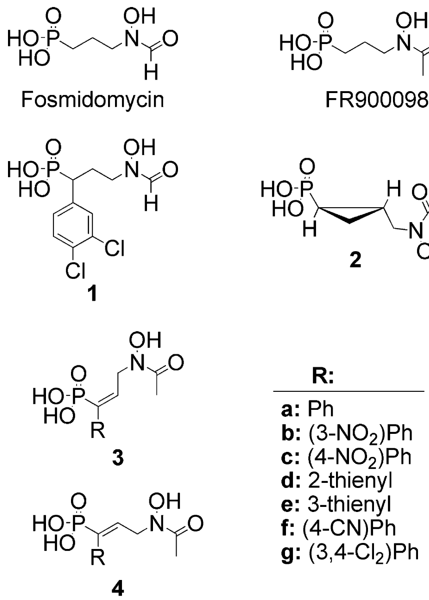


Figure 1. Structures of fosmidomycin, FR900098 and analogues under study.

afforded analogues, which exhibited antiplasmodial activities that came close to that of the FR900098 prodrug, while a 3,4-difluorophenyl-substituted analogue was slightly more potent.[12]

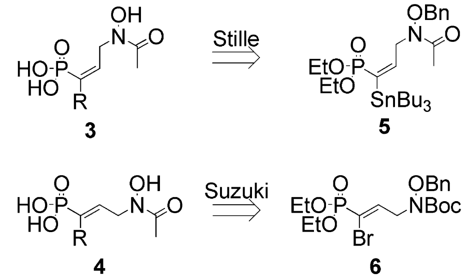
Consistent with our findings, α-aryl-substituted fosmidomycin analogues were generally superior to their FR900098 homologues. The introduction of an ethyl, propyl, isopropyl, dimethyl and hydroxymethyl group was associated with a considerable drop in antimalarial activity.[12] Also α-arylmethyl[13] or phenylethyl[14] analogues failed to surpass the activities of the α-aryl prodrugs.

Furthermore, rigidification of the carbon spacer by the introduction of a cyclopropane[15] (as in **2**) or cyclopentane[11] ring indicated a preferred *trans* geometry for the substituents on these rings for binding DXR.

The scope of the present work is to combine both features, that is, rigidification of the carbon spacer (through incorporation of an α,β-unsaturated bond) and introduction of an α-aryl substituent. As the α,β-double bond could occur both in the *E*- or in the *Z*-configuration, these analogues might provide insight into the preferred binding conformation of saturated α-substituted fosmidomycin analogues.

The retrosynthetic approaches towards the synthesis of the *Z*- and *E*-configured analogues of FR900098 are briefly depicted in Scheme 1. The synthesis of the *Z*-analogues **3a–e** is based on a palladium(0)-catalysed Stille-coupling on organotin derivative **5**,[16] while the synthesis of the *E*-configured analogues **4a**,**f–g** features a Suzuki-type cross-coupling on vinylic bromide **6** as a key-step.

The synthesis of compounds **8a–e** in five steps from THP-protected propargyl alcohol **7** via Stille coupling

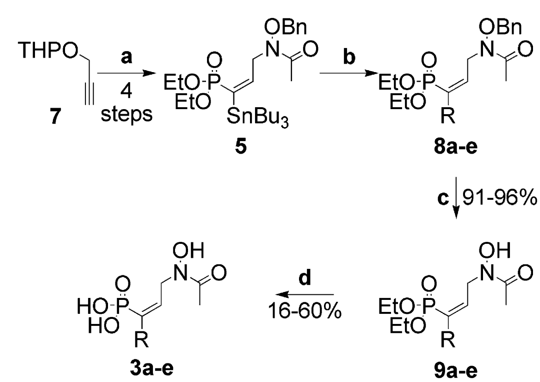


Scheme 1. Retrosynthetic approach towards analogues **3** and **4**.

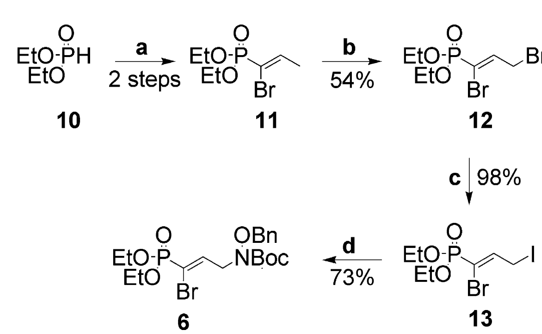
of **5** (Scheme 2) was recently reported as part of a divergent synthetic route towards α-substituted fosmidomycin analogues.[16] Due to the inherent stereoselectivity of the Stille-coupling, only the *Z*-isomers were obtained.

Further elaboration to FR900098 analogues **3a–e** involved a BCl_3-assisted removal of the benzyl protecting group from **8a** to **8e**. Subsequent cleavage of the phosphonate ester groups with TMSBr followed by purification by reversed phase HPLC yielded the *Z*-configured analogues **3a–e** in low to moderate yields.[17]

A Suzuki-based strategy was chosen for the synthesis of the *E*-α,β-unsaturated analogues **4a**,**f–g** (Scheme 3). First *Z*-α-bromo-1-propenyl phosphonate (**11**) was syn-



Scheme 2. Reagents and conditions: (a) see Ref. 16; (b) Ar—I, $Pd_2dba_3 \cdot CHCl_3$, $(2\text{-furyl})_3P$, CuI, NMP, rt; (c) BCl_3, CH_2Cl_2, −50 °C; (d) TMSBr, MeCN, rt; C-18 RP HPLC.

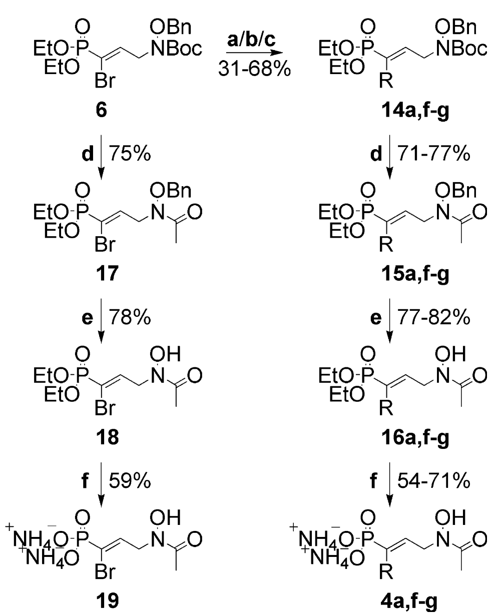


Scheme 3. Reagents and conditions: (a) see Ref. 18; (b) NBS, $Ph(COO)_2$, CCl_4, reflux; (c) NaI, acetone, rt; (d) BnONHBoc, NaH,DMF, rt.

thesized from diethyl phosphite (**10**) in two steps.[18] The required *Z*-configuration was assigned based on the $^3J_{PH}$ coupling constant (14.2 Hz) in the ^{1}H NMR spectrum, which is in accordance with the coupling constant typically found for a vinylic proton located *cis* to a phosphonate.[19] Radical allylic bromination afforded compound **12** in moderate yield. Prior conversion of the allylic bromide **12** into iodide **13** via Finkelstein reaction was required due to the low reactivity of compound **12** in the substitution reaction to yield key intermediate **6**. Conversely, when this substitution reaction was applied to iodide **13**, protected allylic hydroxylamine **6** was obtained in an adequate yield.

Optimization of the Suzuki-coupling with a series of arylboronic acids (Scheme 4) proved to be troublesome, as poor to moderate yields were obtained for **14a**,**f–g**. Furthermore, every analogue required different, specific conditions for the reaction to occur, in contrast to the generally applicable Stille-conditions described above.

To complete the synthesis of analogues **4a**,**f–g**, the Boc-protecting group of **14a**,**f–g** was first removed, followed by in situ acetylation of the free amine to yield the *N*-benzyloxyacetamides **15a**,**f–g**. Subsequent removal of the benzyl protecting group with BCl_3 and cleavage of the phosphonate esters yielded the final *E*-configured α,β-unsaturated, α-aryl substituted analogues **4a**,**f–g**, which were purified via Whatman CF-11 cellulose chromatography, resulting in much improved yields as compared to RP-chromatographic purification used for **3a–e**.[19,20] Furthermore, application of the same deprotection sequence directly on the vinylic bromide **6** also



Scheme 4. Reagents and conditions: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 80 °C; (b) (4-CN)$PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, THF, H_2O, 80 °C; (c) (3,4-Cl_2)$PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, dioxane, H_2O, 80 °C; (d) (i)—TFA, CH_2Cl_2, 0 °C; (ii)—Ac_2O, pyridine, rt; (e) BCl_3, CH_2Cl_2, −50 °C; (f) TMSBr, MeCN, rt; $NH_4OH_{(aq)}$; CF-11 cellulose chromatography.

Table 1. IC_{50} values of the synthesized FR900098 analogues against recombinant *E. coli* DXR

Compound	R	IC_{50} *E. coli* DXR (μM)
Fosmidomycin		0.034
FR900098		0.032
1		0.059
3a	Ph	>30
3b	(3-NO_2)Ph	>30
3c	(4-NO_2)Ph	34
3d	2-Thienyl	42
3e	3-Thienyl	>30
4a	Ph	5.7
4f	(3,4-Cl_2)Ph	5.5
4g	(4-CN)Ph	16
19	Br	0.45

allowed for the synthesis of the *Z*-α,β-unsaturated α-bromo-substituted analogue **19**.

Because of the difficulties associated with the handling of *P. falciparum* DXR, we investigated the ability of the synthesized FR900098 analogues **3a–e**, **4a**,**f–g** and **19** to inhibit the highly homologous *Escherichia coli* isozyme. The conversion of DOXP to MEP by the enzyme was determined in an assay based on the NADPH dependency of the reaction and the results are summarized in Table 1.[21]

Compared to fosmidomycin and FR900098, the *Z*-configured unsaturated analogues **3a–e** were found to be much weaker inhibitors of *E. coli* DXR, which was anticipated considering the previously found preferred *trans*-orientation of the phosphonate and retrohydroxamate functionalities in cyclopropyl and cyclopentyl fosmidomycin analogues.[11,15] Unexpectedly, in contrast with the active *trans*-substituted cyclopropyl derivative **2**, also the *E*-configured α-aryl substituted FR900098 analogues **4a**,**f–g** displayed poor inhibitory activity towards *E. coli* DXR. Apparently, the combination of an α-aryl substituent and an α,β-unsaturated bond constrains the rotational freedom in a way that is unfavourable for binding to the active site of DXR. It should be noted that the relative conformation of two *trans*-substituents on a cyclopropane ring diverges from that of the *trans*-substituents in the *E*-configured analogues **4a**,**f–g**. Remarkably, the α-bromo derivative **19**, capable of undergoing electronical interactions with the target enzyme, performs much better than analogues **3a–e** and **4a**,**f–g**.

In conclusion, two different divergent procedures for the preparation of both *Z*- and *E*-unsaturated α-aryl-substituted FR900098 analogues **3** and **4** were developed using, respectively, a Stille and a Suzuki coupling as a key step. Unfortunately, with the exception of vinylic bromide **19**, none of the synthesized compounds exhibited submicromolar DXR inhibitory activity.

Acknowledgments

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17. Spectral data for **3a**: ^{1}H NMR (300.13 MHz; D_2O) δ 2.09 (s, 3H, —CH_3), 4.74–4.76 (m, 2H, —NCH_2), 5.94 (dt, 1H, J = 40.0 and 5.5 Hz, =C*H*—CH$_2$N), 7.32 (s, 5H, arom. H); ^{13}C NMR (75.47 MHz; D_2O) δ 19.3 (s, —*C*H$_3$), 48.4 (d, $^{3}J_{PC}$ = 6.1 Hz, =CH—*C*H$_2$N), 127.2 (s, arom. =*C*H), 128.1 (d, J_{PC} = 3.8 Hz, 2× arom. =CH), 128.3 (s, 2× arom. =CH), 136.3 (d, $^{2}J_{PC}$ = 7.7 Hz, =*C*H—CH$_2$N), 142.1 (d, $^{2}J_{PC}$ = 11.0 Hz, arom. =C), 142.6 (d, $^{1}J_{PC}$ = 164.1 Hz, P—*C*=CH), 173.5 (s, N—C=O); ^{31}P NMR (121.50 MHz, D_2O) δ 8.9 ppm; HR-MS (ESI-MS) $[M-H]^-$ found, 270.0535; calcd, 270.0531.
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20. *Typical procedure for the preparation and purification of the final ammonium phosphonates demonstrated for* **4a**: To a solution of **16a** (72 mg, 0.219 mmol) in dry MeCN (2.2 mL) was added dropwise TMSBr (290 μL, 3.67 mmol) at rt and the mixture was stirred for 24 h. The solvents were removed under reduced pressure and remaining traces of TMSBr were removed under high vacuum (0.05 mbar). The residual oil was dissolved in 2 mL of Type I water and the pH of the mixture was adjusted to 8–9 with a 5% NH_4OH solution. The solution was lyophilized and the residual solid was purified by Whatman CF-11 cellulose column chromatography [MeCN/NH_4OH (aq, 1 M): 4:1]. The fractions were assayed using cellulose TLC and the spots were visualized under UV-light (365 nm) after dipping in a pinacryptol yellow solution (0.1% in H_2O) and drying the plate under a stream of hot air. The appropriate fractions were lyophilized, yielding 32 mg of a white hygroscopic solid (54%). Spectral data for **4a**: ^{1}H NMR (300.13 MHz; D_2O) δ 1.83 (s, 3H, minor —CH_3), 2.02 (s, 3H, major —CH_3), 4.94 (dd, 2H, J = 6.4 and 2.7 Hz, =CH—CH_2N), 6.35 (dt, 1H, J = 20.6 and 6.6 Hz, major =C*H*—CH$_2$N), 6.42–6.52 (m, 1H, minor =C*H*—CH$_2$N), 7.22 (app dt, 2H, J = 8.0 and 1.6 Hz, arom. H), 7.31–7.40 (m, 3H, arom. H); ^{13}C NMR (75.47 MHz; D_2O) δ 19.2 (s, —*C*H$_3$), 46.7 (d, $^{3}J_{PC}$ = 18.6 Hz, =CH—*C*H$_2$N), 127.5 (d, arom. =*C*H, $^{5}J_{PC}$ = 1.7 Hz), 128.4 (s, 2× arom. =*C*H), 129.0 (d, J_{PC} = 4.4 Hz, 2× arom. =CH), 132.9 (d, $^{2}J_{PC}$ = 9.9 Hz, arom. =C), 136.3 (d, $^{2}J_{PC}$ = 8.8 Hz, =*C*H—CH$_2$N), 142.7 (d, $^{1}J_{PC}$ = 168.0 Hz, P—*C*=CH), 173.7 (s, N—C=O); ^{31}P NMR (121.50 MHz; D_2O) δ 10.5 and 10.8 ppm (minor and major isomer); HR-MS (ESI-MS) $[M-2\times NH_4^{+}+H^{+}]^{-}$found, 270.0535; calcd, 270.0531.
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