UNIVERSITEIT GENT

FACULTY OF **PHARMACEUTICAL SCIENCES**

SYNTHESIS AND EVALUATION OF β-SUBSTITUTED FOSMIDOMYCIN ANALOGUES AS INHIBITORS OF 1-DEOXY-D-XYLULOSE 5-PHOSPHATE REDUCTOISOMERASE

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INTRODUCTION & GOAL

Blocking the MEP pathway for isoprenoid biosynthesis offers interesting prospects for inhibiting *Plasmodia* growth. Fosmidomycin (**1**) and its homologue FR900098 (**2**) potently inhibit 1-deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr), a key enzyme in this pathway. Although fosmidomycin is a remarkably safe antimalarial agent, low oral absorption, short serum half-life and malaria recrudescence preclude its use in monotherapy. The development of more lipophilic Dxr inhibitors able to passively permeate into cells with improved pharmacokinetic properties could lead to more efficacious agents.
Previously, we discovered that analogue **4**, featuring a 3,4-dichlorophenyl substituent in α-position of the phosphonate, surpasses fosmidomycin's potency in inhibiting *P. falciparum* growth. Here we explored the introduction of aryl or aralkyl substituents at the β-position of the known hydroxamate analogue **3**.

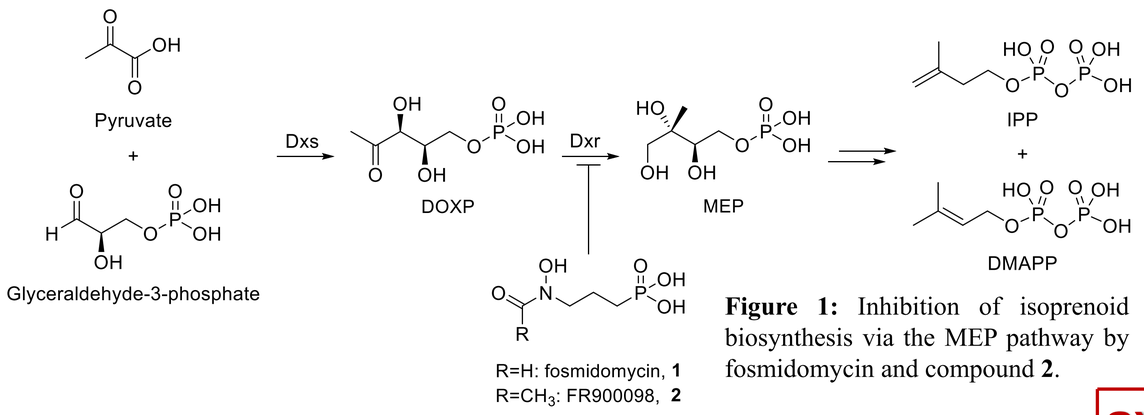
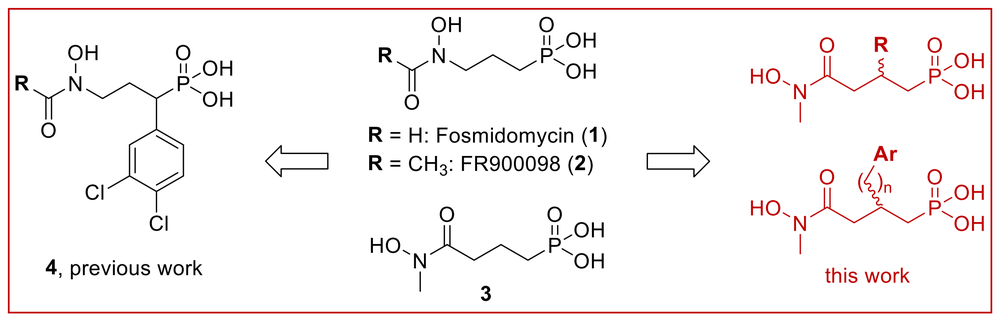
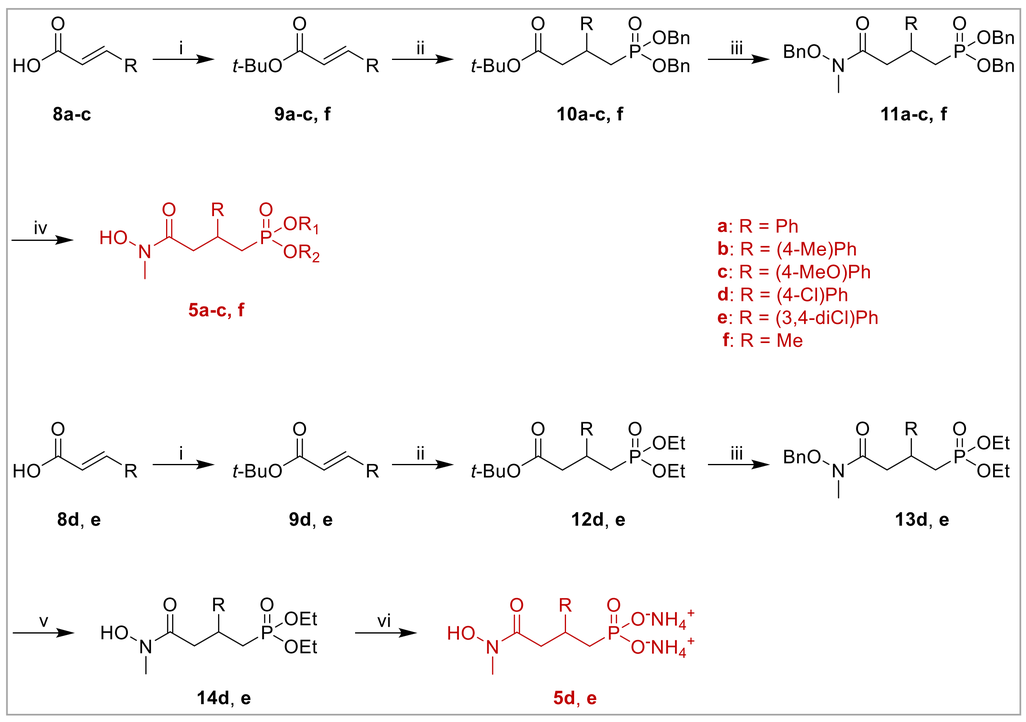


Figure 1: Inhibition of isoprenoid biosynthesis via the MEP pathway by fosmidomycin and compound **2**.



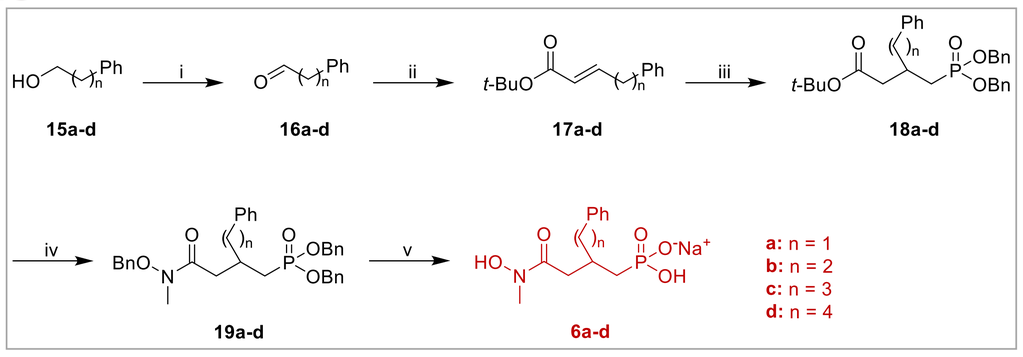
SYNTHESIS

I. Synthesis of β-aryl analogues



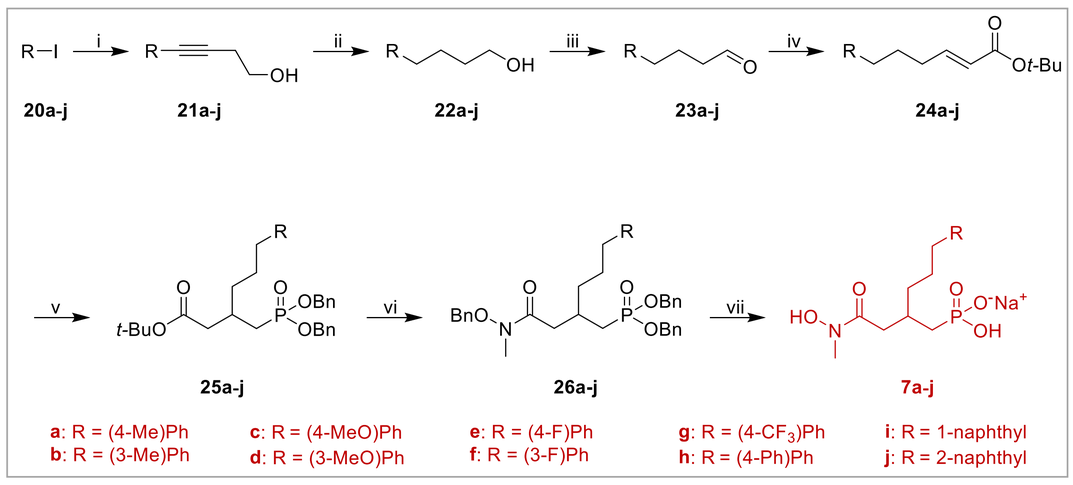
Reagents and conditions: i) Boc_2O, DMAP, *tert*-BuOH, rt, overnight; ii) $(BnO)_2OPMe$ (for **10a-c**, **f**), $(EtO)_2OPMe$ (for **12d** & **12e**), n-BuLi, THF, -78 °C, 2.5 h; iii) (a) TFA, CH_2Cl_2, 45 min, 0 °C to rt; (b) MeNH(OBn), EDC, DMAP, CH_2Cl_2, rt, overnight; iv) H_2, Pd/C, MeOH, NaOHaq., 25 °C, 10–15 min; v) BCl_3, CH_2Cl_2, -78 °C, 1 h; vi) (a) TMSBr, BSTFA, CH_2Cl_2, 0 °C to rt, 22 h; (b) H_2O, NH_4OHaq.

II. Synthesis of β-phenylalkyl homologues



Reagents and conditions: i) Dess-Martin periodinane, CH_2Cl_2; ii) Ph_3P=CHCOOtert-Bu, toluene, 120 °C; iii) $(BnO)_2OPMe$, n-BuLi, THF, -78 °C, 2.5 h; iv) (a) TFA, CH_2Cl_2, 45 min, 0 °C to rt; (b) MeNH(OBn), EDC, DMAP, CH_2Cl_2, rt; v) H_2, Pd/C, MeOH, NaOHaq., 25 °C, 10–15 min.

III. Synthesis of β-arylpropyl homologues



Reagents and conditions: i) but-3-yn-1-ol, $PdCl_2(PPh_3)$, CuI, Et_3N, 117 °C; ii) H_2, Pd/C, MeOH; iii) Dess-Martin periodinane, CH_2Cl_2; iv) Ph_3P=CHCOO*tert*-Bu, toluene, 120 °C; v) $(BnO)_2OPMe$, n-BuLi, THF, -78 °C; vi) (a) TFA, CH_2Cl_2, 45 min, 0 °C to rt; (b) MeN(OBn)H, EDC, DMAP, CH_2Cl_2, 18 h rt; vii) H_2, Pd/C, MeOH, NaOHaq., 25 °C, 10-15 min.

BIOLOGICAL RESULTS

Table 1. IC_{50} ± sd values for recombinant Dxr from *P. falciparum* and MIC_{50} values against *in vitro* growth of *P. falciparum* K1 strain.

Cmpd	PfDxr IC_{50} (μM)	*P. falciparum-K1* MIC_{50} (μM)	Cmpd	PfDxr IC_{50} (μM)	*P. falciparum-K1* MIC_{50} (μM)
Fos (1)	0.036 ± 0.006	1.73 ± 0.89	**6c**	**0.117 ± 0.012**	**0.43 ± 0.09**
FR (2)	0.018	0.42 ± 0.17	**6d**	**0.069 ± 0.005**	**< 0.25±0.00**
3		**0.26 ± 0.02**	**7a**	**0.56 ± 0.007**	2.71
5a	3.3 ± 0.17	>64	**7b**	**0.05 ± 0.007**	14.11
5b	9.3 ± 0.75	>64	**7c**	**0.12 ± 0.006**	6.59
5c	18.8 ± 4.2	>64	**7d**	**0.15 ± 0.006**	10.44
5d		>64	**7e**	3.2 ± 0.115	> 64.00
5e		>64	**7f**	**0.07 ± 0.001**	10.77
5f		**0.74 ± 0.13**	**7g**	**0.27 ± 0.01**	> 64.00
6a		≥56.8 ± 10.1	**7h**	1.6 ± 0.25	> 64,00
6b	1.36 ± 0.02	35.4 ± 8.7	**7i**	**0.28 ± 0.002**	56.01
			7j	**0.87 ± 0.03**	41.24

X-RAY STRUCTURES OF PfDxr IN COMPLEX WITH 6c,d

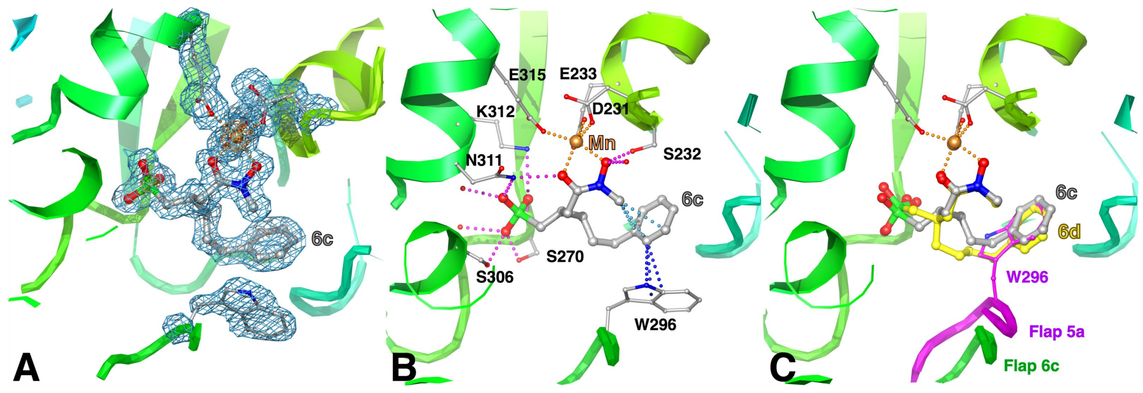


Figure 2. The active site of PfDxr bound to **6c** and **6d**. Water molecules are shown as small red spheres, and the Mn^{2+} ion is gold.
(A) Electron density for the inhibitor **6c** and selected nearby residues. (B) Hydrogen-bond interactions (magenta) between **6c** and protein or solvent and metal coordination (gold); blue and cyan bubbles indicate close contacts (< 3.7 Å) between the phenyl group of **6c** and the indole ring of Trp296 from the flap, or within the inhibitor, respectively. (C) The structures of bound **6c** and **6d** are superimposed. The flap in the complex with **5a** is shown in magenta for comparison.

CONCLUSIONS

We studied the effect of introducing substituents in β-position of the hydroxamate analogue **3**. While direct addition of a β-aryl moiety resulted in poor *P. falciparum* Dxr inhibition, longer linkers between the carbon backbone and the phenyl ring were generally associated with better binding to the enzyme. X-ray structures of the parasite Dxr-inhibitor complexes show that the "longer" compounds generate a substantially different flap structure, in which a key tryptophan residue is displaced, and the aromatic group of the ligand lies between the tryptophan and the hydroxamate's methyl group. Several analogues emerged as highly potent inhibitors of *Plasmodium falciparum in vitro* growth. In some cases (*e.g.* for compounds **7b** and **7f**) good Dxr inhibitory activity failed to translate in good *in vitro* activity against the parasite, which may be due to inefficient uptake. Compounds **5a-e** likewise failed to inhibit EcDxr and MtbDxr while **6c** was optimal for inhibition of these enzymes.