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# Synthesis and Biological Evaluation with Plant Cells of New Fosmidomycin Analogues Containing a Benzoxazolone or Oxazolopyridinone Ring

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**Fosmidomycin, 3-(*N*-formyl-*N*-hydroxyamido) propylphosphonic acid sodium salt, is an efficient inhibitor of 1-deoxy-D-xylulose-5-phosphate (DOXP) reductoisomerase, the second enzyme of the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway notably present in *Plasmodium* species. We have synthesized a new series of analogues of fosmidomycin, containing a benzoxazolone, benzoxazolethione or oxazolopyridinone ring. As the MEP pathway is involved in the biosynthesis of all isoprenoids, accumulation of ajmalicine in *Catharanthus roseus* cells was chosen as a marker of monoterpenoid indole alkaloid (MIA) production. None of the twelve studied phosphonic esters 3 and phosphonic acids 4 affected periwinkle cell growth, but some of them (3c, 3e, 3g and 3h) showed a significant inhibition of ajmalicine accumulation: 45–85% at 125 μM. Surprisingly, this effect disappeared by conversion of 3c and 3g into the corresponding acids 4c and 4g, respectively.**

*Keywords*: Catharanthus roseus; Monoterpenoid indole alkaloids production; Benzoxazolone, benzoxazolethione derivatives, oxazolopyridinone derivatives; Fosmidomycin analogues

## INTRODUCTION

Malaria is one of the most serious tropical diseases causing between 1.5 and 2.7 million fatal cases per year. Nearly all fatal cases are caused by *Plasmodium falciparum*, one of the causative agents of *Malaria tropica*. This is largely due to the widespread emergence of *P. falciparum* strains that became resistant to the presently available drugs. As a result, there is an urgent need for new efficient antimalarial agents.

Recently, the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway, firstly discovered in bacteria, was demonstrated to be present in algae, plants and several Apicomplexa species including *Plasmodium falciparum*. The MEP pathway provides isopentenyl diphosphate (IPP), the common precursor of all isoprenoids, and it was proven that the natural antibiotic fosmidomycin is an efficient inhibitor of the second enzyme of the MEP pathway, i.e. 1-deoxy-D-xylulose-5-phosphate (DOXP) reductoisomerase.[1,2] Enzymes of this pathway represent targets for the search of new classes of herbicides and antimalarial drugs.

Indeed, fosmidomycin and its derivative FR-900098 (Figure 1) have herbicide activities[3,4] and protect mice against *Plasmodium vinckei* infection.[5,6] This first successful application of herbicides acting on the MEP pathway as antimalarial drugs[7] has been recently confirmed and fosmidomycin was shown to be an effective treatment for malaria.[8]

The aim of the present work concerns the synthesis of substrate analogues based on the fosmidomycin structure in order to elucidate some steps and find inhibitors of the MEP pathway in a model plant cell culture, *Catharanthus roseus*. We investigated the effect of fosmidomycin used as control and found that the drug had no effect on periwinkle cell growth but inhibited the accumulation of monoterpenoid indole alkaloids (MIA): production of ajmalicine was

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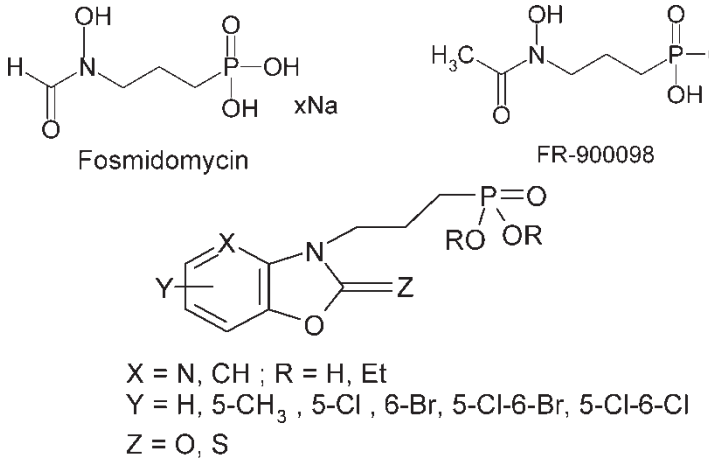



FIGURE 1 Structures of fosmidomycin, FR-900098 and analogues.



inhibited in a dose-dependent manner with an $IC_{50}$ value of 10 μM.[9] One pot synthesis of functionalized 4,5-dihydroisoxazole derivatives *via* nitrile oxides and biological evaluation with plant cells was previously described.[9]

Benzoxazolone or oxazolopyridinone and their biologically active derivatives have been the object of numerous studies aimed at establishing their potential applications as drugs and pesticides.[10–12] Their activities depend mainly on the nature of the substituents on the basic heterocyclic framework. It is known that halogen-substituted 2-benzoxazolones have significant antibacterial and fungicidal properties.[13] For this reason, investigating the biological properties of compounds containing benzoxazolone or oxazolopyridinone ring could lead to the discovery of new representatives of this class having valuable pharmacological properties against malaria. This paper deals with the preparation of new fosmidomycin analogues containing a benzoxazolone or oxazolopyridinone ring and their biological evaluation on *C. roseus* cell growth and MIA accumulation.

## MATERIALS AND METHODS

### Chemistry

#### *Instrumentation*

$^1H$ and $^{13}C$ NMR spectra of diethyl phosphonates **3** and phosphonic acids **4** were recorded on a Bruker DPX 200 at 200.131 MHz and 50.32 MHz, respectively, in deuteriochloroform and $D_2O$. The coupling constants are recorded in hertz (Hz) and the chemical shifts are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Mass spectra were recorded on a R 10-10 C Nermag (70 eV) apparatus. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60 F 254, Merck), and spots were visualized under UV light or by iodine vapor. Flash chromatography was performed with Kieselgel 60 (230–400 mesh) silica gel (Merck).

#### *Reagents and Chemicals*

Organic solvents were purified when necessary according to literature methods[14] or purchased from Aldrich Chimie. All solutions were dried over anhydrous magnesium sulfate and evaporated on a Buchi rotatory evaporator. All anhydrous reactions were performed in oven-dried glassware under an argon atmosphere. The column chromatography solvents employed were distilled and solvent mixtures were reported as volume to volume ratios. Starting materials were purchased from Aldrich Chimie (St Quentin-Fallavier, France) or Acros (Noisy-le-Grand, France). Literature procedures were used for the preparation of 2(3*H*)-benzoxazolone,[15,16] 5-methyl-2(3*H*)-benzoxazolone,[17] 5-chloro-2(3*H*)-benzoxazolone[18,19] and 5, 6-halosubstituted derivatives[20]. Oxazolo[4,5-*b*]pyridin-2(3*H*)-one[21] and 6-bromooxazolo[4,5-*b*]pyridin-2(3*H*)-one were prepared according to the reported procedures.[22] 6-Chloro-2-benzoxazolethiol was furnished by Acros. Diethyl 3-bromopropyl-phosphonate[23] was prepared by the method of Arbusov in a 76% yield. Fosmidomycin and fosmidomycin diethyl ester were prepared according to published procedures.[24]

#### *Synthesis*

Alkylation of Benzoxazolones, 6-Chlorobenzoxazolethiol or Oxazolopyridinones by Diethyl 3-bromopropylphosphonate;

*General Procedure for the Preparation of Esters **3a–h**.* To a freshly prepared solution of sodium ethoxide (76 mg of sodium in 10 ml of ethanol) was

slowly added compound **2** (3 mmol). The mixture was stirred for 1 h at room temperature, then the ethanol was evaporated. To the resulting mixture in DMF (15 ml) was added dropwise a solution of diethyl 3-bromopropylphosphonate (855 mg, 3.3 mmol) in DMF (2 ml). The mixture was refluxed (110°C) overnight. After cooling and evaporation of the solvent under reduced pressure, the residue was washed with water and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was chromatographed on a silica gel column with $CH_2Cl_2$/MeOH (95:5) as eluent to give the desired pure oil.

**3-[2-(Diethoxyphosphoryl)propyl]-2(3*H*)-Benzoxazolone (3a)**

The title compound was prepared from benzoxazolone according to the general procedure; yellow oil; yield: 71%. $^1$H NMR ($CDCl_3$) δ 1.08 (6H, t, J = 7.02 Hz, C**H$_3$**CH$_2$O), 1.59 (2H, m, PC**H$_2$**), 1.87 (2H, m, C**H$_2$**CH$_2$P), 3.71 (2H, t, J = 6.86 Hz, N—C**H$_2$**), 3.87 (4H, quintet, J = 7.56 Hz, CH$_3$C**H$_2$**O), 6.85–6.94 (4H, m, Harom). $^{13}$C NMR ($CDCl_3$) δ 16.58, 16.70 ($J_{PC}$ = 6.0 Hz, POCH$_2$**C**H$_3$), 21.38, 21.47 ($J_{PC}$ = 4.5 Hz, PCH$_2$**C**H$_2$), 24.31 (P**C**H$_2^*$), 42.60, 42.27 ($J_{PC}$ = 16.6 Hz, N—**C**H$_2$), 61.84, 61.97 ($J_{PC}$ = 6.5 Hz, PO**C**H$_2$CH$_3$), 108.67, 110.08, 122.64, 124.12, 130.50, 131.16, 142.73 (Carom), 154.58 (**C**═O). MS (CI with $NH_3$) m/z 314 (M + 1). (*) One line of the P**C**H$_2$-doublet disturbed by the POCH$_2$CH$_3$ signal.

**5-Methyl-3-[2-(diethoxyphosphoryl)propyl]-2(3*H*)-benzoxazolone (3b)**

The title compound was prepared from 5-methylbenzoxazolone according to the general procedure; yellow oil; yield: 68%. $^1$H NMR ($CDCl_3$) δ 1.35 (6H, t, J = 7.06 Hz, C**H$_3$**CH$_2$O), 1.83 (2H, m, PC**H$_2$**), 2.08 (2H, m, C**H$_2$**CH$_2$P), 2.42 (3H, s, C**H$_3$**), 3.93 (2H, t, J = 6.99 Hz, N—C**H$_2$**), 4.13 (4H, quintet, J = 7.76 Hz, CH$_3$C**H$_2$**O), 6.88–7.30 (3H, m, Harom). $^{13}$C NMR ($CDCl_3$) δ 16.81, 16.93 ($J_{PC}$ = 6.0 Hz, POCH$_2$**C**H$_3$), 21.60, 21.69 ($J_{PC}$ = 4.5 Hz, PCH$_2$**C**H$_2$), 21.82, 24.67 ($J_{PC}$ = 143.4 Hz, P**C**H$_2$), 42.48, 42.82 ($J_{PC}$ = 17.1 Hz, N—**C**H$_2$), 62.13, 62.26 ($J_{PC}$ = 6.5 Hz, PO**C**H$_2$CH$_3$), 109.32, 110.08, 123.32, 131.28, 134.37, 141.06, (Carom), 155.27 (**C**═O). MS (CI with $NH_3$) m/z 329 (M + 1).

**5-Chloro-3-[2-(diethoxyphosphoryl)propyl]-2(3*H*)-benzoxazolone (3c)**

The title compound was prepared from 5-chlorobenzoxazolone according to the general procedure; yellow oil; yield: 68%. $^1$H NMR ($CDCl_3$) δ 1.36 (6H, t, J = 7.07 Hz, C**H$_3$**CH$_2$O), 1.83 (2H, m, PC**H$_2$**), 2.11 (2H, m, C**H$_2$**CH$_2$P), 3.94 (2H, t, J = 7.10 Hz, N—C**H$_2$**), 4.13 (4H, quintet, J = 7.58 Hz, CH$_3$C**H$_2$**O), 7.08–7.18 (3H, m, Harom). $^{13}$C NMR ($CDCl_3$) δ 16.81, 16.93 ($J_{PC}$ = 6.0 Hz, POCH$_2$**C**H$_3$), 21.54, 21.64 ($J_{PC}$ = 5.0 Hz, PCH$_2$**C**H$_2$), 24.57 (P**C**H$_2^*$), 42.97, 42.66 ($J_{PC}$ = 15.6 Hz, N—**C**H$_2$), 62.36, 62.23 ($J_{PC}$ = 6.5 Hz, PO**C**H$_2$CH$_3$), 109.35, 111.33, 122.86, 129.96, 132.39, 141.50, (Carom), 154.73 (**C**═O). MS (CI with $NH_3$) m/z 348 (M + 1). (*) One line of the P**C**H$_2$-doublet disturbed by the POCH$_2$CH$_3$ signal.

**5,6-Dichloro-3-[2-(diethoxyphosphoryl)propyl]-2(3*H*)-benzoxazolone (3d)**

The title compound was prepared from 5,6-dichlorobenzoxazolone according to the general procedure; yellow oil; yield: 70%. $^1$H NMR ($CDCl_3$) δ 1.29 (6H, t, J = 7.04 Hz, C**H$_3$**CH$_2$O), 1.75 (2H, m, PC**H$_2$**), 2.04 (2H, m, C**H$_2$**CH$_2$P), 3.89 (2H, t, J = 7.00 Hz, N—C**H$_2$**), 4.07 (4H, quintet, J = 7.11 Hz, CH$_3$C**H$_2$**O), 7.19, 7.27 (2H, two s, Harom). $^{13}$C NMR ($CDCl_3$) δ 16.73, 16.85 ($J_{PC}$ = 6.0 Hz, POCH$_2$**C**H$_3$), 21.51, 21.42 ($J_{PC}$ = 4.5 Hz, PCH$_2$**C**H$_2$), 24.36 (P**C**H$_2^*$), 42.98, 42.69 ($J_{PC}$ = 14.6 Hz, N—**C**H$_2$), 62.28, 62.15 ($J_{PC}$ = 6.5 Hz, PO**C**H$_2$CH$_3$), 110.30, 112.34, 126.39, 128.20, 131.10, 141.60, (Carom), 154.15 (**C**═O). MS (CI with $NH_3$) m/z 383 (M + 1). (*) One line of the P**C**H$_2$-doublet disturbed by the POCH$_2$CH$_3$ signal.

**6-Bromo-5-chloro-3-[2-(diethoxyphosphoryl)-propyl]-2(3*H*)-benzoxazolone (3e)**

The title compound was prepared from 5-chloro-6-bromobenzoxazolone according to the general procedure; yellow oil; yield: 74%. $^1$H NMR ($CDCl_3$) δ 1.31 (6H, t, J = 7.05 Hz, C**H$_3$**CH$_2$O), 1.82 (2H, m, PC**H$_2$**), 2.06 (2H, m, C**H$_2$**CH$_2$P), 3.90 (2H, t, 7.04 Hz, N—C**H$_2$**), 4.09 (4H, quintet, J = 7.19 Hz, CH$_3$C**H$_2$**O), 7.22, 7.42 (2H, two s, Harom). $^{13}$C NMR ($CDCl_3$) δ 16.79, 16.91 ($J_{PC}$ = 6.0 Hz, POCH$_2$**C**H$_3$), 21.55, 21.45 ($J_{PC}$ = 5.0 Hz, PCH$_2$**C**H$_2$), 24.41 (P**C**H$_2^*$), 42.72, 43.02 ($J_{PC}$ = 15.1 Hz, N—**C**H$_2$), 62.21, 62.34 ($J_{PC}$ = 6.5 Hz, PO**C**H$_2$CH$_3$), 110.27, 115.20, 115.31, 130.14, 131.78, 141.76, (Carom), 154.15 (**C**═O). MS (CI with $NH_3$) m/z 426 ($^{79}$Br), 428 ($^{81}$Br). (*) One line of the P**C**H$_2$-doublet disturbed by the POCH$_2$CH$_3$ signal.

**3-[2-(Diethoxyphosphoryl)propyl]-oxazolo-[4,5-*b*]pyridin-2(3*H*)-one (3f)**

The title compound was prepared from oxazolo-[4,5-*b*]pyridin-2(3H)-one according to the general procedure; yellow oil; yield: 61%. $^1$H NMR ($CDCl_3$) δ 1.34 (6H, t, J = 7.00 Hz, C**H$_3$**CH$_2$O), 1.86 (2H, m, PC**H$_2$**), 2.20 (2H, m, C**H$_2$**CH$_2$P), 4.01–4.19 (6H, m, CH$_3$C**H$_2$**O +N—C**H$_2$**), 7.06–8.15 (3H, m, Harom). $^{13}$C NMR ($CDCl_3$) δ 16.81, 16.92 ($J_{PC}$ = 5.5 Hz, POCH$_2$**C**H$_3$), 21.72, 21.81 ($J_{PC}$ = 4.5 Hz, PCH$_2$**C**H$_2$), 22.20, 25.05 ($J_{PC}$ = 143.0 Hz, P**C**H$_2$), 41.72, 42.11 ($J_{PC}$ = 19.6 Hz, N—**C**H$_2$), 62.22, 62.09 ($J_{PC}$ = 6.5 Hz, PO**C**H$_2$CH$_3$), 116.60, 118.66, 137.35, 143.63, 145.94 (Carom), 153.74 (**C**═O). MS (CI with $NH_3$) m/z 315 (M + 1).

**6-Bromo-3-[2-(diethoxyphosphoryl)propyl]-oxazolo[4,5-*b*]pyridin-2(3*H*)-one (3g)**

The title compound was prepared from 6-bromooxazolo[4,5-*b*]pyridin-2(3H)-one according to the general procedure; yellow oil; yield: 52%. $^1$H

NMR ($CDCl_3$) δ 1.34 (6H, t, J = 7.05 Hz, **C**$H_3CH_2O$), 1.83 (2H, m, PC**H$_2$**), 2.18 (2H, m, C**H$_2$**$CH_2$P), 3.99–4.19 (6H, m, $CH_3$C**H$_2$**O + N—C**H$_2$**), 7.60 (d, $J_{7,5}$ = 1.84 Hz, 1H, H-7), 8.21 (d, $J_{5,7}$ = 1.84, 1H, H-5). $^{13}$C NMR ($CDCl_3$) δ 16.80, 16.91 ($J_{PC}$ = 5.5 Hz, POC$H_2$**C**$H_3$), 21.59, 21.68 ($J_{PC}$ = 4.5 Hz, PC$H_2$**C**$H_2$), 22.16, 25.02 ($J_{PC}$ = 144.0 Hz, P**C**$H_2$), 41.92, 42.30 ($J_{PC}$ = 19.1 Hz, N—**C**$H_2$), 62.13, 62.26 ($J_{PC}$ = 6.5 Hz, PO**C**$H_2$CH$_3$), 113.56, 119.78, 137.43, 144.30, 144.83 (Carom), 153.21 (**C**═O). MS (CI with $NH_3$) m/z 393 ($^{79}$Br), 395 ($^{81}$Br).

**6-Chloro-3-[2-(diethoxyphosphoryl)propyl]-2(3*H*)-benzoxazolethione (3h)**

The title compound was prepared from 6-chloro-2-benzoxazolethiol according to the general procedure; yellow oil; yield: 75%. $^1$H NMR ($CDCl_3$) δ 1.16 (6H, t, J = 7.00 Hz, C**H$_3$**$CH_2O$), 1.84 (2H, m, PC**H$_2$**), 2.03 (2H, m, C**H$_2$**$CH_2$P), 3.25 (2H, t, J = 6.81 Hz, N—C**H$_2$**), 3.96 (4H, m, $CH_3$C**H$_2$**O), 7.06–7.33 (3H, m, Harom.). $^{13}$C NMR ($CDCl_3$) δ 16.66, 16.77 ($J_{PC}$ = 5.5 Hz, POC$H_2$**C**$H_3$), 22.93, 23.01 ($J_{PC}$ = 4.0 Hz, PC$H_2$**C**$H_2$), 23.36, 26.19 ($J_{PC}$ = 142.0 Hz, P**C**$H_2$), 33.04, 32.69 ($J_{PC}$ = 17.6 Hz, N—**C**$H_2$), 62.02, 61.89 ($J_{PC}$ = 6.5 Hz, PO**C**$H_2$CH$_3$), 110.76, 119.00, 125.08, 129.71, 140.93, 152.15 (Carom), 165.58 (**C**═O). MS (CI with $NH_3$) m/z 364 (M + 1).

*General Procedure for the Hydrolysis of the Diethyl Phosphonates **3** to Phosphonic Acids **4**.* Trimethylsilyl bromide (536 mg, 3.5 mmol) was added to a solution of the diethyl phosphonate **3** (0.5 mmol) in dry methylene chloride (3 ml) under ice-bath cooling. The mixture was stirred at room temperature overnight and then concentrated under reduced pressure to leave an oil. This oil was dissolved in water (3 ml) and stirred at room temperature for 1 h. The solution was washed with chloroform (2 × 2 ml) and treated with activated charcoal. After removal of the charcoal by filtration, the filtrate was concentrated under reduced pressure. The residue was dissolved into water (2 ml) and adjusted to pH 4.0–5.0 with 1M NaOH. The mixture was concentrated under reduced pressure to give a residue, which was dissolved in methanol, and ethanol was added to the solution. The mixture was stirred overnight at room temperature; then the precipitated crystalline solid was collected and recrystallized from methanol-ethanol to give the corresponding phosphonic acid as the monosodium salt.

**[3-(2-Oxo-2(3*H*)-benzoxazol-3-yl)propyl]phosphonic Acid (4a)**

Yellow oil; yield: 80%. $^1$H NMR (DMSO-$d_6$) δ 1.63 (2H, m, PC**H$_2$**), 1.88 (2H, m, C**H$_2$**$CH_2$P), 3.90 (2H, t, J = 6.55 Hz, N—C**H$_2$**), 7.11–7.38 (4H, m, Harom). $^{13}$C NMR (DMSO-$d_6$) δ 22.39 (PC$H_2$**C**$H_2$), 24.15, 26.88 ($J_{PC}$ = 137.4 Hz, P**C**$H_2$), 43.15, 42.78 ($J_{PC}$ = 18.6 Hz, N—**C**$H_2$), 110.03, 110.56, 123.09, 124.79, 131.96, 142.81 (Carom), 154.63 (**C**═O). MS (CI with $NH_3$) m/z 258 (M + 1).

**[3-(2-Oxo-5-methyl-2(3*H*)-benzoxazol-3-yl)propyl]-phosphonic Acid (4b)**

Yellow oil; yield: 76%. $^1$H NMR (DMSO-$d_6$) δ 1.58 (2H, m, PC**H$_2$**), 1.88 (2H, m, C**H$_2$**$CH_2$P), 2.52 (3H, s, $CH_3$), N—$CH_2$-overlapping with $H_2O$ in DMSO-$d_6$, 6.93–7.24 (3H, m, Harom). $^{13}$C NMR (DMSO-$d_6$) δ 21.91 (**C**$H_3$), 22.36 (PC$H_2$**C**$H_2$), 24.16, 26.89 ($J_{PC}$ = 137.4 Hz, P**C**$H_2$), 42.76, 43.13 ($J_{PC}$ = 18.6 Hz, N—**C**$H_2$), 110.15, 110.40, 123.38, 131.83, 134.35, 140.82 (Carom), 154.92 (**C**═O). MS (CI with $NH_3$) m/z 272 (M + 1).

**[3-(2-Oxo-5-chloro-2(3*H*)-benzoxazol-3-yl)propyl]-phosphonic Acid (4c)**

Yellow oil; yield: 70%. $^1$H NMR (DMSO-$d_6$) δ 1.57 (2H, m, PC**H$_2$**), 1.84 (2H, m, C**H$_2$**$CH_2$P), 3.86 (2H, t, N—C**H$_2$**), 7.14–7.56 (3H, m, Harom). $^{13}$C NMR (DMSO-$d_6$) δ, 22.36 (PC$H_2$**C**$H_2$), 24.04, 26.77 ($J_{PC}$ = 137.4 Hz, P**C**$H_2$), 43.03, 43.39 ($J_{PC}$ = 18.1 Hz, N—**C**$H_2$), 110.31, 111.85, 122.67, 128.98, 133.37, 141.60 (Carom), 154.59 (**C**═O). MS (CI with $NH_3$) m/z 292 (M + 1).

**[3-(2-Oxo-oxazolo[4,5-*b*]pyridin-2(3*H*)-3-yl)-propyl]phosphonic Acid (4f)**

Yellow oil; yield: 95%. $^1$H NMR ($D_2O$) δ 1.77 (4H, m, PC**H$_2$** + C**H$_2$**$CH_2$P), 3.82 (2H, t, 6.38 Hz, N—C**H$_2$**), 7.05–7.92 (3H, m, Harom). $^{13}$C NMR ($D_2O$) δ 21.08 (PC$H_2$**C**$H_2$), 22.45, 25.17 ($J_{PC}$ = 136.9 Hz, P**C**$H_2$), 42.23, 41.83 ($J_{PC}$ = 20.1 Hz, N—**C**$H_2$), 118.82, 119.41, 137.68, 141.49, 144.75 (Carom), 155.03 (**C**═O). MS (CI with $NH_3$) m/z 259 (M + 1).

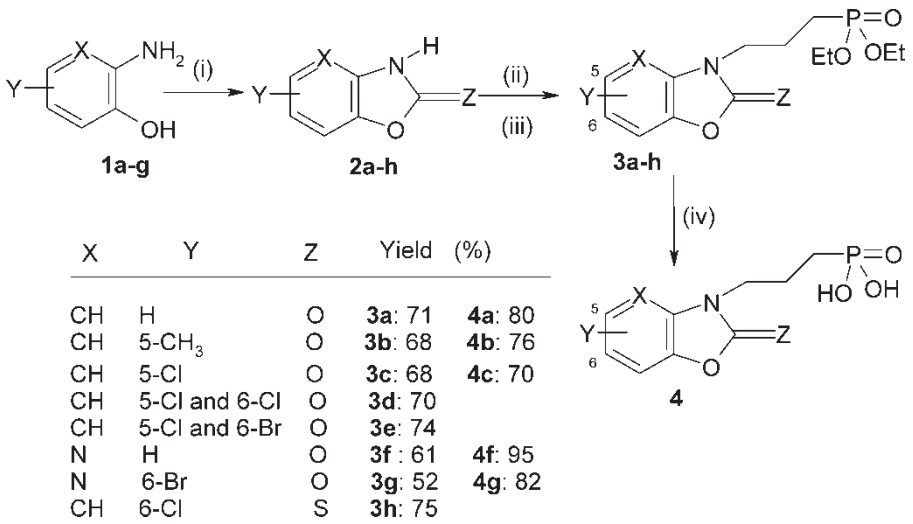
**[3-(2-Oxo-6-bromooxazolo[4,5-*b*]pyridin-2(3*H*)-3-yl)propyl]phosphonic Acid (4g)**

Yellow oil; yield: 82%. $^1$H NMR (DMSO-$d_6$) δ 1.60 (2H, m, C**H$_2$**$CH_2$P), 1.93 (2H, m, P—C**H$_2$**), N—C**H$_2$**-overlapping with $H_2O$ in DMSO-$d_6$, 8.08 (s, 1H, H-7), 8.26 (s, 1H, H-5). $^{13}$C NMR ($D_2O$) δ 22.21 (PC$H_2$**C**$H_2$), 22.45, 25.17 ($J_{PC}$ = 136.9 Hz, P**C**$H_2$), N—**C**$H_2$-overlapping DMSO-$d_6$, 113.10, 120.11, 137.88, 143.72, 145.70 (Carom), 153.49 (**C**═O). MS (CI with $NH_3$) m/z 337, 339.

## Biological Evaluation

### *Plant Material*

Periwinkle (*Catharanthus roseus* [L.] G. Don) cell suspensions (line C20) were subcultured every 7 days (dilution rate 1: 10) in B5 Gamborg medium[25] containing 58 mM sucrose and 4.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D)[26]. They were grown in 250 ml Erlenmeyer flasks (with 50 ml culture medium) on a rotary shaker (100 rpm) at 24°C, in darkness.[27]




| X | Y | Z | Yield (%) | |
|---|---|---|---|---|
| CH | H | O | **3a**: 71 | **4a**: 80 |
| CH | 5-$CH_3$ | O | **3b**: 68 | **4b**: 76 |
| CH | 5-Cl | O | **3c**: 68 | **4c**: 70 |
| CH | 5-Cl and 6-Cl | O | **3d**: 70 | |
| CH | 5-Cl and 6-Br | O | **3e**: 74 | |
| N | H | O | **3f** : 61 | **4f**: 95 |
| N | 6-Br | O | **3g**: 52 | **4g**: 82 |
| CH | 6-Cl | S | **3h**: 75 | |

SCHEME 1 Synthesis of esters **3** and acids **4**. Reagents: (i) $NH_2CONH_2$, reflux. (ii) EtONa, EtOH, r.t. (iii) diethyl 3-bromopropylphosphonate, DMF, reflux. (iv) $(CH_3)_3SiBr$, $CH_2Cl_2$, 0°C.

### *Treatments*

Periwinkle cells were subcultured in a MIA-inducing medium (i.e. 2,4-D-free B5 medium to which 4.5 μmol zeatin was added at day 3).[28] The molecules to be tested were added at day 3 to the medium (final concentrations 50, 75, 100 and 125 μM). The cells were harvested at day 7 (vacuum filtration) and freeze dried for ajmalicine and dry mass quantitations.

### *Alkaloid Quantitation*

MIA were extracted from 25 mg freeze dried cells with methanol. Ajmalicine, the chosen marker of alkaloid accumulation, was quantified by spectrofluorodensitometry[27] (TLC scanner 3, Camag, λex: 365 nm, λem :> 400 nm).

## RESULTS AND DISCUSSION

### Chemistry

Benzoxazolones **2a–e**, pyridoxazolones **2f–g** prepared from the corresponding *ortho*-aminophenols, were used as starting materials for the synthesis of the fosmidomycin ester analogues **3a–h**. Alkylation was achieved by heating **2** overnight with diethyl 3-bromopropylphosphonate in refluxing DMF, in the presence of sodium ethoxide. The reaction products were purified by column chromatography. Using the described procedure, eight diethyl phosphonates **3**, containing a benzoxazolone or oxazolopyridinone ring were synthesized in good yields. The purity and the identity of the products were confirmed by $^1H$ and $^{13}C$ NMR and MS.

The phosphonic acid **4a–g** can be prepared by hydrolyzing the corresponding diethyl phosphonates **3a–g** (Scheme 1). The hydrolysis includes a combination method comprising transformation of the diethyl ester into a silyl ester and its subsequent hydrolysis in water.[24] The hydrolysis of the diethyl phosphonates **3** is usually carried out in the presence or absence of solvents under anhydrous conditions and cooling.[29–31] Trimethylbromosilane (used in an amount of 5 or more molar equivalents) smoothly and quantitatively converts a variety of dialkyl phosphonates to the corresponding bis(trimethylsilyl)esters under very mild conditions, typically 1 h at 25°C in good yield.[24,29,30]

### Biology

Firstly, we investigated the effects of the studied fosmidomycin analogues on the cell growth of

TABLE I Effects of the studied compounds at 125 μM on ajmalicine content of *C. roseus* cells

| Compounds | Ajmalicine production (mg/g DW)[a] |
|---|---|
| **3a** | 3.10 ± 0.01 (+55) |
| **3b** | 2.00 ± 0.01 |
| **3c** | 0.60 ± 0.02 (− 70) |
| **3d** | n.t. [b] |
| **3e** | 0.40 ± 0.03 (− 80) |
| **3f** | 2.00 ± 0.01 |
| **3g** | 1.10 ± 0.01 (− 45) |
| **3h** | 0.60 ± 0.01 (− 70) |
| **4a** | 3.16 ± 0.01 (+55) |
| **4b** | 3.01 ± 0.02 (+50) |
| **4c** | 2.00 ± 0.01 |
| **4f** | 2.00 ± 0.01 |
| **4g** | 2.00 ± 0.01 |

[a] DW: dry weight. Values are the mean of three determinations ± SE. Percentage of modification of alkaloid accumulation is given in parentheses. Ajmalicine production in control cells: 2.00 ± 0.03 mg/g DW. [b] n.t.: not tested, non-soluble compound in culture medium.

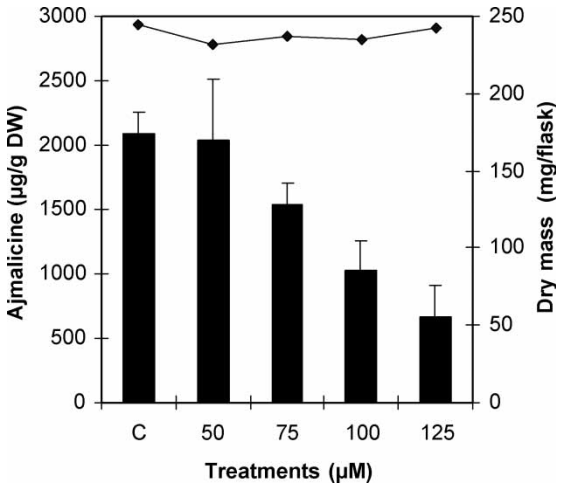



FIGURE 2 Effect of ester **3c** on alkaloid production in *C. roseus* cells (■). Cell growth is not affected (—◆—). Data represent average values from three replicates ± SE. DW = dry weight. C = control cells.

*Catharanthus roseus*. Interestingly, up to 125 µM, no compound disturbed the cell proliferation; there was no significant difference with the control cell growth.

Secondly, we studied their capacity to modify MIA production in periwinkle cells (Table I). It was previously established that fosmidomycin diethyl ester and diethyl 3-(4,5-dihydroisoxazol-3-yl)ethyl-phosphonates, compared to fosmidomycin, increased MIA production. In the present case, it was observed (Table I) that among seven phosphonates only one compound, **3a**, increased ajmalicine production; two others, **3b** and **3f**, had no effect and four, **3c**, **3e**, **3g**, **3h** (Figure 2), showed an inhibitory effect: 45–85% at 125 µM. Surprisingly, none of the five studied phosphonic acids inhibited MIA accumulation: stimulation by **3a** was maintained on ajmalicine production in its acid **4a** (Figure 3), and an increase was observed with **4b**; hydrolysis of **3f** into **4f** induced no change and, lastly, the inhibitory effect exerted by **3c** and **3g** disappeared on conversion into the corresponding acids **4c** and **4g**.

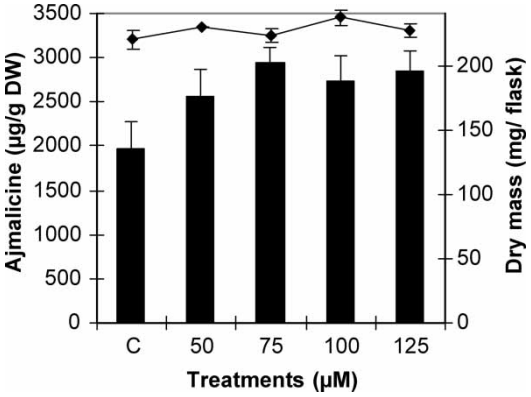



FIGURE 3 Effect of acid **4a** on alkaloid production in *C. roseus* cells cell (■). Cell growth is not affected (—◆—). Data represent average values from three replicates ± SE. DW = dry weight. C = control cells.

## CONCLUSION

Although it requires caution to deduce definite conclusions from this limited series of fosmidomycin analogues, it seems that the presence of halogen(s), positioned at the six-membered ring of the heterocycle, exerts a favourable effect as far as inhibition of MIA production is concerned. To our knowledge, this is the first time that inhibition of MIA accumulation has been observed with fosmidomycin analogues. This encouraging result tends to confirm that these compounds could present a potential interest for the treatment of malaria.

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