

Efficient Method for the Synthesis of Diazaphospholidines: Toxicological Evaluation

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Abstract In this work, we described the synthesis of new heterocyclic organophosphorus compounds starting from a primary amine and phenyl phosphonic dichloride (PDCP). The introduction of a phosphoryl group into heterocyclic structures can generate potential chemical and biological activities. We have prepared the phosphoramidates in only one step. These compounds provide access to diazaphospholidine by intermolecular cyclization using dibromoethane. We chose primary amines (cyclohexylamine, benzylamine, propylamine and phenyl ethylamine) to prepare phosphoramidates. The toxicological activity of two synthesized molecules was evaluated.

Keywords Phenyl Phosphonic Dichloride, Phosphoramidate, Diazaphospholidine

1. Introduction

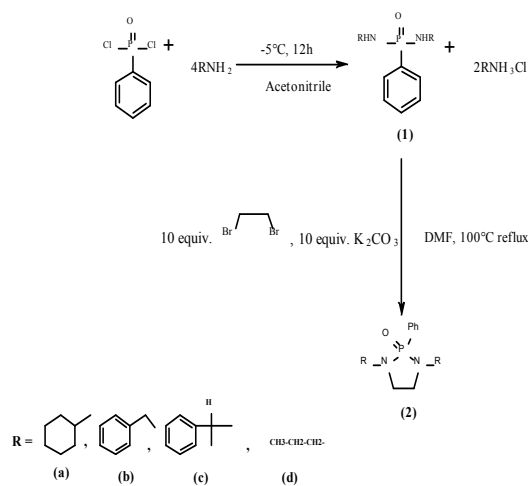
Organophosphorus compounds are ubiquitous in nature and find applications in the field of agriculture, medicine and industry.^{1,2} Some organophosphorus compounds have been described in the literature as inhibitors of bacterial³, herbicides, insecticides, pesticides⁴, antifungal agents⁵, anti-HIV⁶, anti-cancer⁷, antiviral and anti-inflammatory⁸. An important group of this class is phosphoramidate, which have been used in many reactions and synthesis of organic compounds.

A number of research groups has become interested in organophosphorus heterocyclic compounds since they are finding extensive use as pesticides in agriculture, as stabilizers in polymers and as lubricant oil additives. An important family of this heterocycles is diazaphospholidine, which have been used in many reactions serve as ligands for transition metal complexes⁹ phosphorus ligands containing alternative donor units, such as phosphites¹⁰ or diazaphospholidines,¹¹ have been less widely examined.

Herein, we report the synthesis of some novel diazaphospholidine derivatives (**2a-d**), and their corresponding precursors phosphoramidates (**1a-d**). choose to use phenylphosphonic dichloride to introduce phosphoramidate moiety. The toxicological activity of this compounds was evaluated.

2. Results and discussion

The phosphoramidates derivatives (**1a-d**), (Scheme 1) were prepared in a one-pot synthetic route, starting from corresponding primary amines with phenyl phosphonic dichloride in dry acetonitrile at -5°C. These compounds were obtained in good yields. The synthesis of diazaphospholidine was achieved by mixing 10 equivalents of dibromoethane with phosphoramidate in the presence of potassium carbonate. The heterocyclic compounds were obtained as a white solid in 67 % yields.



Scheme 1

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We describe the inhibitory effect of phosphoramidate on

Paramecium Aurelia (1a) and (1b), the unicellular ciliate, *Paramecium* (Peniculia, Oligohymenophorea), is one of the most studied protists. Considerable research has been conducted on its morphology, physiology, genetics, nuclear reorganization, phylogeny, and the concept of 'species' [12].

these ciliates are excellent alternative models in toxicology. Our results showed a perturbation of respiratory metabolism, thus confirming the effect of these molecules on the respiratory chain of micro-organism.

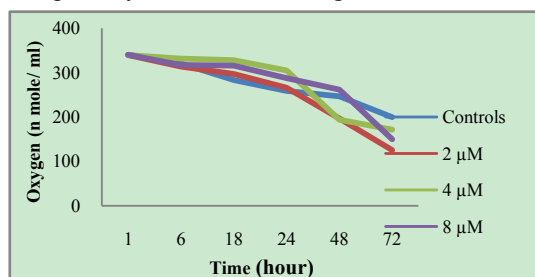


Figure 1. Effects of the molecule (1a) on the respiratory metabolism

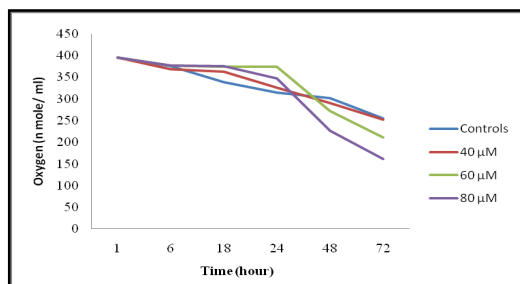


Figure 2. Effects of the molecule (1b) on the respiratory metabolism

The Figure 1 shows the effects of compound **1a** on the parametia metabolism respiratory at concentrations 2, 4 and 8 μM . We note a slight inhibition of O_2 consumption of parametia treated with different concentrations of 1a reflecting a sharp slowdown of their respiratory activity between 6 and 24 hours. Beyond 24 hours, breathing of parametia treated is strongly stimulated.

We note that the cells treated with different concentrations of 1b present a sharp slowdown in their respiratory activity between 6 and 24 hours (figure 2). Beyond 24 hours, the respiration of parametia treated is strongly stimulated.

3. Experimental Section

Melting points were determined in open capillary tubes on an Electro thermal apparatus and uncorrected. IR spectra were recorded on a perkin-Elmer FT-600 spectrometer. Proton nuclearmagnetic resonance was determined with a 360 WB or AC 250-MHz Bruker spectrometer using CDCl_3 and $\text{DMSO}-d_6$ as a solvent and TMS as an internal standard. Chemical shifts are reported in δ units (ppm). All coupling constants (J) are reported in Hertz. Multiplicity is indicated as s (singlet), d (doublet), t (triplet), m (multiplet) and combination of these signals. Electron ionization mass spectra (30 ev) were recorded in positive mode on a Water MicroMass ZQ. High-resolution mass were measured on a

Joel SX 102 mass spectrometer and recorded in FAB positive mode. All reactions were monitored by TLC on silica Merck h60 F254 (Art. 5554) precoated aluminum plates and were developed by spraying with ninhydrin solution.

3.1. Synthesis of Phenyl Phosphoramidates

To a stirred solution of phenyl phosphonic dichloride (0.71 mL, 5 mmol) in dry acetonitrile (35 mL), a solution of primary amine (1.98 g, 20 mmol, 4 equiv) was added dropwise at -5°C . After 12 h, the solvent was evaporated under vacuum and the oily product was washed with distilled water. The organic layer was dried over sodium sulfate and concentrated. The products were purified by column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1). The phosphoramidates were obtained as a white solid in excellent yields.

Bis (cyclohexylamino) phenylphosphine oxide **1a**: Yield: 89%. $M = 320 \text{ g/mol}$ [$\text{C}_{18}\text{H}_{29}\text{N}_2\text{OP}$]. Mp. $156-158^\circ\text{C}$. $R_f = 0.625$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$). IR (KBr, cm^{-1}): 3155.3 cm^{-1} (NH), 1461.9 cm^{-1} ($\text{C}=\text{C}_{\text{Ar}}$), 1180.4 cm^{-1} ($\text{P}=\text{O}$), 1110.9 cm^{-1} ($\text{C}-\text{N}-\text{P}$). ^1H NMR (CDCl_3 , 250MHz): 7.90 (m, 2H, H-Ar); 7.50 (m, 3H, H-Ar); 3.15 (m, 2H, CH-cyc); 2.40 (d, $J = 9.3 \text{ Hz}$, 2H, NH-P=O); 1.90 (m, 4H, CH_2 -cyc); 1.65 (m, 4H, CH_2 -cyc); 1.15 (m, 12H, CH_2 cycl). ^{31}P NMR (CDCl_3 , 200.7MHz) $\delta = 16.42 \text{ ppm}$. ^{13}C NMR (CDCl_3 , 250MHz): 134 (CH-Ar); 132 (CH-Ar); 129 (CH-Ar); 55 (CH-Hex); 35(CH_2 -Hex); 25 (CH_2 - Hex); 24.8 (CH_2 - Hex).

Bis (benzylamino) phenylphosphine oxide **1b**: Yield: 91%. $M = 336 \text{ g/mol}$ [$\text{C}_{20}\text{H}_{21}\text{N}_2\text{OP}$]. Mp. $159-161^\circ\text{C}$. $R_f = 0.60$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$). IR (KBr, cm^{-1}): 3163 cm^{-1} (NH), 1461.9 cm^{-1} ($\text{C}=\text{C}_{\text{Ar}}$), 1253.6 cm^{-1} ($\text{P}=\text{O}$), 1118.6 cm^{-1} ($\text{C}-\text{N}-\text{P}$). ^1H NMR (CDCl_3 , 250MHz): 7.9 (m, 2H, H-Ar); 7.5 (m, 3H, H-Ar); 7.25 (m, 10H, H-Ar); 4.15 (d, $J = 6.85 \text{ Hz}$, 4H, CH_2 -N); 3.0 (m, 2H, NH-P=O). ^{31}P NMR (CDCl_3 , 200.7MHz) $\delta = 16.33 \text{ ppm}$. ^{13}C NMR (CDCl_3 , 250MHz): 141 (C-Ar); 134.1 (C-Ar); 134 (CH-Ar); 132 (CH-Ar); 129 (CH-Ar); 128.5 (CH-Ar); 127 (CH-Ar); 126.6 (CH-Ar); 44 (CH_2).

Bis (phenylethylamino) phenylphosphine oxide **1c**: Yield: 87.15%. $M = 364 \text{ g/mol}$ [$\text{C}_{22}\text{H}_{25}\text{N}_2\text{OP}$]. Mp. $164-167^\circ\text{C}$. $R_f = 0.63$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$). IR (KBr, cm^{-1}): 3209.3 cm^{-1} (NH), 1450.4 cm^{-1} ($\text{C}=\text{C}_{\text{Ar}}$), 1191.9 cm^{-1} ($\text{P}=\text{O}$), 1029.9 cm^{-1} ($\text{C}-\text{N}-\text{P}$). ^1H NMR (CDCl_3 , 250MHz): 7.75 (m, 2H, H-Ar); 7.4 (m, 3H, H-Ar); 7.35-7 (m, 10H, H-Ar); 4.60 (m, 1H, *CH-NH); 4.35 (m, 1H, *CH-NH); 2.75 (s, 2H, NH-P=O); 1.5 (d, $J = 6.25 \text{ Hz}$, 3H, CH_3); 1.3 (d, $J = 6.77 \text{ Hz}$, 3H, CH_3). ^{31}P NMR (CDCl_3 , 200.7MHz) $\delta = 16.65 \text{ ppm}$. ^{13}C NMR (CDCl_3 , 250MHz): 145 (C-Ar); 134.2 (C-Ar); 132.1 (CH-Ar); 130 (2 C, CH-Ar); 128.8 (CH-Ar); 128.5 (CH-Ar); 126.9 (CH-Ar); 126.7 (CH-Ar); 55 (CH); 24 (CH_3).

Bis (propylamino) phenylphosphine oxide **1d**: Yield: 88.53%. $M = 240 \text{ g/mol}$ [$\text{C}_{12}\text{H}_{21}\text{N}_2\text{OP}$]. Mp. $150-153^\circ\text{C}$. $R_f = 0.617$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$). IR (KBr, cm^{-1}): 3193.9 cm^{-1} (NH), 1465.8 cm^{-1} ($\text{C}=\text{C}_{\text{Ar}}$), 1200 cm^{-1} ($\text{P}=\text{O}$), 1033.8 cm^{-1} ($\text{C}-\text{N}-\text{P}$). ^1H NMR (CDCl_3 , 250MHz): 7.9 (m, 2H, H-Ar);

7.5 (m, 3H, H-Ar); 2.9 (m, 4H, CH₂-NH); 2.5 (m, 2H, NH-P=O); 1.5 (m, 4H, CH₂-CH₃); 0.9 (t, $J_1 = 7.31$ Hz, $J_2 = 7.46$ Hz, 6H, CH₃). ³¹P NMR (CDCl₃, 200.7MHz) $\delta = 16.4$ ppm. ¹³C NMR (CDCl₃, 250MHz): 135 (C-Ar); 131.5 (CH-Ar); 128.3 (CH-Ar); 128.30 (CH-Ar); 42.5(CH₂-NH); 25.3 (CH₂-CH₃); 11.3 (CH₃).

3.2. Synthesis of Diazaphospholidine

A solution of phenyl phosphoramidates (1 g, 3 mmol, 1 equiv) and excess of K₂CO₃ (4.14 g, 30 mmol, 10 equiv) in DMF (2 mL) was stirred, a solution of 1, 2- dibromoethane (2.59 g, 30 mmol, 10 equiv) was added dropwise. The reaction mixture was refluxed for 2h. The residue was evaporated in vacuum. The products were purified by column chromatography on silica gel (CH₂Cl₂/MeOH 9/1) afforded diazaphospholidines as a white solid in 67 % yields.

1,3-cyclohexyl, 2-phenyl diazaphospholidin-2-oxide **2a**: Yield: 66.78%. M = 346 g /mol [C₂₀H₃₁N₂OP]. Mp. 171-173°C. $R_f = 0.83$ (CH₂Cl₂/MeOH). IR (KBr, cm⁻¹): 1451.5 cm⁻¹ (C=C_{Ar}), 1181.2 cm⁻¹ (P=O), 1109.4 cm⁻¹ (C-N-P). ¹H NMR (CDCl₃, 250MHz): 7.95 (m, 2H, H-Ar); 7.55 (m, 3H, H-Ar); 3.20 (m, 2H, CH-cyc); 2.5 (t, $J_1 = 9.4$ Hz, $J_2 = 9.42$ Hz, 4H, CH₂-cyc à 5); 1.95 (m, 4H, CH₂-cyc); 1.70 (m, 4H, CH₂-cyc); 1.20 (m, 12H, CH₂-cycl). ³¹P NMR (CDCl₃, 200.7MHz) $\delta = 33.55$ ppm. ¹³C NMR (CDCl₃, 250MHz): 134.2 (C-Ar); 131.9 (1 C, CH-Ar); 130.3 (CH-Ar); 128.8 (CH-Ar); 60 (CH-Hex); 44.1 (CH₂-cyc); 33.1 (CH₂-Hex); 25.7 (CH₂-Hex); 25.1 (CH₂-Hex).

1, 3-benzyl, 2-phenyl diazaphospholidin-2-oxide **2b**: Yield: 69.42%. M = 346g/mol [C₂₂H₃₁N₂OP]. Mp. 174-177 °C. $R_f = 0.81$ (CH₂Cl₂/MeOH). IR (KBr, cm⁻¹): 1460.1 cm⁻¹ (C=C_{Ar}), 1253.4 cm⁻¹ (P=O), 1117.6 cm⁻¹ (C-N-P). ¹H NMR (CDCl₃, 250MHz): 7.95 (m, 2H, H-Ar); 7.55 (m, 3H, H-Ar); 7.30 (m, 10H, H-Ar); 4.20 (d, $J = 6.95$ Hz, 4H, CH₂-N); 3.0 (t, $J_1 = 8.8$ Hz, $J_2 = 8.9$ Hz, 4H, CH₂cyc à 5). ³¹P NMR (CDCl₃, 200.7MHz) $\delta = 33.34$ ppm.

¹³C NMR (CDCl₃, 250MHz): 136.4 (C-Ar); 134.2 (C-Ar); 134.1 (CH-Ar); 132.3 (CH-Ar); 128.8 (CH-Ar); 128.5 (CH-Ar); 127.9 (CH-Ar); 127(CH-Ar); 73.3 (CH₂); 45.6 (CH₂).

1, 3-phenylethyl, 2-phenyl, diazaphospholidin-2-oxide **2c**: Yield: 64.29%. M = 390g/mol [C₂₄H₂₇N₂OP]. Mp. 178-179°C. $R_f = 0.80$ (CH₂Cl₂/MeOH). IR (KBr, cm⁻¹): 1458.1 cm⁻¹ (C=C_{Ar}), 1184.2 cm⁻¹ (P=O), 1107.1 cm⁻¹ (C-N-P). ¹H NMR (CDCl₃, 250MHz): 7.75 (m, 2H, H-Ar); 7.5 (m, 3H, H-Ar); 7.4-7.0 (m, 10H, H-Ar); 4.70 (m, 1H, *CH-NH); 4.40 (m, 1H, *CH-NH); 2.8 (t, $J_1 = 9.34$ Hz, $J_2 = 9.48$ Hz, 2H, CH₂-cyc à 5); 2.7 (t, $J_1 = 8.82$ Hz, $J_2 = 8.57$ Hz, 2H, CH₂-cyc à 5); 1.45 (d, $J = 6$ Hz, 3H, CH₃); 1.30 (d, $J = 6.76$ Hz, 3H, CH₃). ³¹P NMR (CDCl₃, 200.7MHz) $\delta = 34.64$ ppm. ¹³C NMR (CDCl₃, 250MHz): 138.3 (C-Ar); 134.2 (C-Ar); 134.1 (CH-Ar); 132.3 (CH-Ar); 128.8 (CH-Ar); 128.5 (CH-Ar); 127.9 (CH-Ar); 127 (CH-Ar); 45.6 (CH); 43.4 (CH₂); 20.8 (CH₃).

1, 3-propyl, 2-phenyl diazaphospholidin-2-oxide **2d**: Yield: 68.31%. M = 266 g /mol [C₁₄H₂₃N₂OP]. Mp. 168-170°C. $R_f = 0.82$ (CH₂Cl₂/MeOH). IR (KBr, cm⁻¹): 1451.4 cm⁻¹ (C=C_{Ar}), 1192.9 cm⁻¹ (P=O), 1029.3 cm⁻¹ (C-N-P). ¹H NMR (CDCl₃, 250MHz): 7.80 (m, 2H, H-Ar); 7.65 (m, 3H, H-Ar); 3.0 (m, 4H, N-CH₂-CH₂); 2.5 (t, $J_1 = 9.37$ Hz, $J_2 = 9.43$ Hz, 4H, CH₂ cyc à 5); 1.55 (m, 4H, -CH₂-CH₃); 0.95 (t, $J_1 = 9.35$ Hz, $J_2 = 9.49$ Hz, 6H, CH₃). ³¹P NMR (CDCl₃, 200.7MHz) $\delta = 33.7$ ppm. ¹³C NMR (CDCl₃, 250MHz): 134.6 (C-Ar); 131.5 (CH-Ar); 128.3 (CH-Ar); 128.3 (CH-Ar); 77 (CH₂-NH); 42.57(CH₂); 25.29 (CH₂); 11.28 (CH₃).

4. Conclusions

This work is based essentially on the synthesis of a new family of modified diazaphospholidines. We achieved the synthesis of phenyl phosphoramidates derived from primary amines (cyclohexylamine, benzylamine, propylamine, and ethylphenylamine) with phenyl phosphonic dichloride in one step. Our toxicological results showed a perturbation of respiratory metabolism, confirming the effect of these molecules on the respiratory chain. Thus we can conclude that the xenobiotics tested are cytotoxic. This toxicity is manifested by the loss of linearity of the trajectory and mobility followed by an inhibition of cell growth.

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