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SYNTHESIS OF OXAZOLIDIN-2-ONES PHOSPHONATES DERIVATIVES: TOXIC EFFECT ON PARAMECIUM SPECIES

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ABSTRACT

A new series of substituted oxazolidin-2-ones containing chloroacetyl and phosphonate groups have been synthesized and their *in vitro* cytoxicity activities were evaluated against *Paramecium sp* at different parameters. Their synthesis were easily carried out starting from available oxazolidin-2-ones by chloroacetylation reaction following by introduction of phosphonate group using $P(OEt)_3$ via Arbuzov reaction. Toxicological impact of these synthetic compounds showed promising results.

Keywords: Heterocyclic, Oxazolidin-2-one, Chloroacetylation, Arbuzov Reaction, Toxicology *Paramecium*

INTRODUCTION

Heterocyclic compounds are a very interesting class for organic synthesis because of their diverse and potent biological properties[1]. Chiral oxazolidin-2-ones derivatives, in particular, have been described to exhibit a wide range of biological properties[2]. Also, they have been widely used as chiral auxiliaries in many asymmetric synthesis[3], as protecting groups in organic synthesis[4], as ligands for metal catalysts[5], as antimicrobial agents[6-7], and as building blocks in polymers. In the field of bioactive molecules, organophosphorus compounds have received a great deal of attention[8]. The introduction of phosphonate group in simple ring such as Aziridine[9], Pyrroles[10]and Pyrazole[11] could be very interesting because they can be functional substrates for the preparation of biologically active compounds. Phosphonate analogs possess a non-hydrolysable C-P band in place of labile O-P band in organophosphate esters, and such present this possibility of antimetabolic activity[12]. In the spite of their importance, only a few examples of acyloxazolidinone-phosphonate derivatives could be found in the literature[13-16]. More recently, we described the amidophosphonates[17], modified sulfamides and cyclosulfamides containing phophonate moieties[18]. In the continuity of this research, we report in this paper the synthesis and toxic effect of acyloxazolodin-2-ones phosphonate derivatives on *Paramecium* species: Growth kinetics.

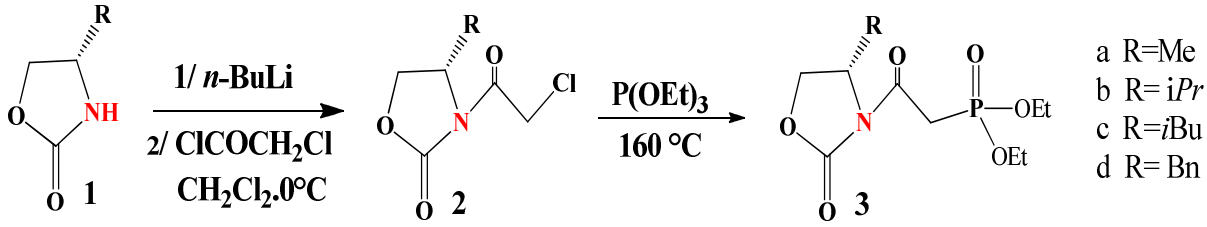
EXPERIMENTAL

All reagents and solvents were of commercially quality and used without further purification. 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 nuclear magnetic resonance was determined with a AC 250-MHz Brüker spectrometer using $CDCl_3$ and 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 (singulet), d (doublet), t (triplet), m (multiplet) and combination of these signals. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values werewithin the acceptable limits of the calculated values. All reactions were monitored by TLC on silica Merck 60 F254 (Art. 5554) pre-coated aluminum plates and were developed by spraying with ninhydrin solution. Column chromatographies were performed on Merck silica gel (230-400 mesh)

RESULTS AND DISCUSSION

The chiral oxazolidinon-2-ones can be prepared in two steps starting from the corresponding (L) amino acids (Ala, Val, Leu, Phe), reduction with sodium borohydride, and cyclization using diethyl carbonate[19]. The starting chiral *N*-chloroacetyl oxazolidin-2-ones (**2a–2d)** were easily prepared in excellent yield (80–90%) by treatment of the corresponding commercially available or easy accessible chiral oxazolidin-2-ones (**1a-1d**) with *n*-BuLi in (CH_2Cl_2) followed by chloroacetylation using chloroacetyl chloride.

The structure of all different *N*-chloroacetyloxazolidin-2-ones (**2a-2d)** were unambiguously confirmed by usual spectroscopic methods. The different ^{1}H NMR spectra showed a signal (singulet) at 4.7-4.8 ppm corresponding to C**H**$_2$Cl protons. These compounds exhibited characteristic absorption in the IR spectrum with the absorption at1718–1728 cm^{-1} (C=O)$_{amide}$ and showed band at 1782-1800 cm^{-1}(C=O)$_{cyle,}$, suggesting its electrophilic ability[20]. The next step is phosphorylation of the (**2a-2d)** in the Michaelis–Arbuzov[21] reaction conditions. The introduction of phosphonate moiety was performed using triethylphosphite. The reaction of chloracetyloxazolidin-2-ones with 2 equiv. of triethylphosphite at 130-150 °C gave the corresponding phosphonates (**3a-3d)** after 10 hours of stirring (Scheme 1). The phosphonates derivatives were obtained with moderate yields 45-52 % after purification on column silica gel. The structure of the (**3a-3d)** was confirmed by ^{1}H NMR and IR. The different ^{1}H NMR showed two caracetistic signal, triplet and quadruplet at 1-1.2 ppm and 4.2-4.4 ppm corresponding at ethyl groupement for phophonate moiety**.**(**3a-3d**) exibited characteristic absorption in the IR spectrum with the absorption at 1248-1262 cm^{-1} (P=O). Elemental (C,H,N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%).



Scheme 1: Preparation of the (3a-3d)

General procedure for the preparation of (S)-4-Alkyl/Benzyl-N-chloroacetyl-oxazolidin-2-ones (2a-2d))

A solution of oxazolidin-2-one (1 mmol) in dry dichloromethane (5mL) and *n*-BuLi (1.1 mmol) were taken in 50 ml round bottom flask under nitrogen, the solution is cooled to 0°C over 30 min. A solution of chloroacetyl chloride (2.2 mmol) in the same solvent was added drop wise at 0°C for 60 min. the resulting mixture was then stirred at room temperature overnight. The organic layer was dried over anhydrous sodium sulfate and removed under reduced pressure. The crude product is purified by silica gel column chromatography eluted with (DCM -MeOH-9.5:0.5) to give white solid.

(S)-4-Methyl-*N*-chloroacetyl-oxazolidin-2-one (2a)

Yield: 90%. White solid.m.p.76-78°C. FT-IR (KBr, ν cm^{-1}): 1665 (C=O $_{amide}$), 1785 (C=O$_{cycle}$), 775 (C-Cl).^{1}H NMR (250 MHz, $CDCl_3$, δ ppm): 1.60 (d, *J* = 6.1 Hz, 3H, CH_3), 4.35 (m, 2H, C**H**$_2$-CH*), 4.55(s, 2H, C**H**$_2$-Cl), 4.30 (m, 1H, C**H***).^{13}C NMR ($CDCl_3$, δ ppm): 169.2, 150.2, 70.0, 65.2, 56.1, 19.2. Calcd. for $C_6H_8NO_3Cl$: C, 40.56; H, 4.50; N, 7.88; Found; C, 40.29; H, 4.39; N, 7.80.

(S)-4-Isopropyl-*N*-chloroacetyl-oxazolidin-2-one (2b)

Yield: 82 %. White solid**.** m.p. 98-100 °C. FT-IR (KBr,ν cm^{-1}): 1668 (C=O $_{amide}$), 1790 (C=O$_{cycle}$), 700-800 (C-Cl). ^{1}H NMR (250 MHz, $CDCl_3$, δppm):1.00-0.93- (2d, *J*= 9Hz, 6H, 2C**H**$_3$), 2.48 (m, 1H, C**H**-(CH_3)$_2$) 4.30 (m, 2H, C**H**$_2$-CH*),4.20 (m, 1H, C**H***), 4.60 (s, 2H, C**H**$_2$-Cl). ^{13}C NMR ($CDCl_3$, δ ppm):

172.2, 152.8, 68.2, 64.7, 63.3, 29.3, 19.3.16.2. Calcd. for $C_8H_{12}NO_3Cl$: C, 46.71; H, 5.83; N, 6.81.Found; C, 46.79; H, 5.85; N, 6.80.

(S)- 4- Isobutyl *N*-chloroacetyl-oxazolidin-2-one (2c)

Yield: 80%. White solid. m.p. 112-114 °C. FT-IR (KBr, ν cm^{-1}): 1670 (C=O $_{amide}$), 1793 (C=O_{cycle}), 771 (C-Cl).^{1}H NMR (250 MHz, $CDCl_3$, δ ppm): 0.95-1.10 (2d, *J* = 9.0 Hz, 6H, 2(C**H**$_3$)$_2$, 1.70 (m, 2H,CH*-C**H**$_2$-CH), 2.10 (m, 1H, C**H**-$(CH_3)_2$), 4.20 (m, 1H,C**H***), 4.50 (m, 2H, O-C**H**$_2$),4.80 (s, 2H, C**H**$_2$-Cl). ^{13}C NMR ($CDCl_3$, δ ppm): 168.4, 150.1, 68.8, 64.6, 61.1, 41.1, 24.5, 23.2, 21.2. Calcd.for $C_9H_{14}NO_3Cl$: C, 49.20; H, 6. 37; N, 6.37. Found: C, 49.29; H, 6.35; N, 6.40.

(S)-4-Benzyl-*N*-chloroacetyl-oxazolidin-2-one (2d)

Yield: 88%. White crystal. m.p. 68-70°C. FT-IR (KBr, ν cm^{-1}): 1682 (C=O $_{amide}$), 1780 (C=O_{cycle}), 774 (C-Cl). ^{1}H NMR (250 MHz, $CDCl_3$, δ ppm): 2.80-3.20(ddd, *J* =13.4, 9.5 , 3.2 Hz, 2H, C**H**$_2$-Ph), 4.20-4.30 (m, 2H, O-C**H**$_2$), 4.36 (m, 1H, C**H***), 4.75 (s, 2H, C**H**$_2$-Cl), 7.20-7.38 (m, 5H, Ar**H**).^{13}C NMR ($CDCl_3$, ppm):169.1, 151.2,138.5, 129.3, 128, 127.5,126.2, 67.1, 65.4, 43.7. Calcd.for $C_{12}H_{12}NO_3Cl$: C, 56.80; H, 4.73; N, 5.52. Found: C, 56.85; H, 4.67; N, 5.50.

General procedure for the preparation of (S)-4-alkyl/benzyl-N-diethylphosphoryl acetyl-oxazolidin-2-one (3a-3d)

The triethylphosphite (4.25mL, 5 eq, 4.96 mmol) and *N*-chloroacetyloxazolidin-2-one were heated at (160 ◦C) under argon. The resulting solution was heated at reflux for an addition 4 hours. The undesired product was removed by a short distillation system. The reaction was monitored with TLC, who indicates the total disappearance of the starting product and the appearance of a product revealed to the molybdenum blue. The crude compound was purified by column chromatography (dichloromethane/methanol, 9/1) to afford the corresponding *N*-4-alkyl/benzy-*N*-diethylphosphoryl-acetyl-oxazolidin-2-one in moderate yield. 48-42 %

(S)-4-Methyl-*N*-diethylphosphoryl-acetyl-oxazolidin-2-one (3a)

Yield: 45%. Yellow oil. FT-IR (KBr,ν cm^{-1}): 1262 (P=O), 1005 (P-O), 1648 (C=O $_{amide}$), 1782.5 (C=O_{cycle}). ^{31}P NMR (200 MHz, $CDCl_3$, δppm): 16 .5.^{1}H NMR (250 MHz, $CDCl_3$,δ ppm): 1.34 (t, *J* = 7.1 Hz, 6H, (C**H**$_3$CH$_2$)$_2$P), 1.60 (d, *J*= 6.1 Hz, 3H, C**H**$_3$), 2.82 (d, $J_{H/P}$ =3.1 Hz, 1 H, C**H**$_2$P), 4.05 (q, *J* = 7.1 Hz , 4H, (CH$_3$C**H**$_2$O) , 4.65 (m, 2H, C**H**$_2$-CH*), 4.15 (m, 1H, C**H***),^{13}C NMR ($CDCl_3$, δ ppm): 168.3, 152.2, 68.7, 61.5, 56.4, 41.8, 18.7, 16.3.Calcd.for $C_{10}H_{18}NO_6P$: C, 43.01; H, 6.45 N, 5.01. Found: C, 43.05; H, 4.47; N, 5.06.

(S)-4-Isopropyl-*N*-diethylphosphoryl-acetyl-oxazolidin-2-one (3b

Yield: 48%. Yellow oil. FT-IR (KBr, ν cm^{-1}): 1252 (P=O), 1008 (P-O),1649.1 (C=O $_{amide}$), 1792.5 (C=O_{cycle}). ^{31}P NMR (200 MHz, $CDCl_3$, δ ppm): 16 .50.^{1}H NMR (250 MHz, $CDCl_3$,δ ppm): 0.87 (d, *J* = 6.9Hz, 3H, CH-(C**H**$_3$)$_2$), 0.82 (d, *J* = 6.9 Hz, 3H, CH-(C**H**$_3$)$_2$), 1.36 (t, *J*= 7.1Hz, 3H, (OCH$_2$-C**H**$_3$)$_2$, 2.30 (m, 1H, C**H**-$(CH_3)_2$), 2.84 (d, $J_{H/P}$ = 3.2, 2H,C**H**$_2$ -P), 4.12 (q, *J* = 7.1 Hz, 4H, (CH$_3$-C**H**$_2$-O)$_2$, 4.30 (m, 1H, C**H***), 4.57 (m, 2H,C**H**$_2$-CH*). ^{13}C NMR ($CDCl_3$, δ ppm): 170.2, 154.3, 65.5, 63.4, 61.5, 42,1, 37.8, 19.3, 17.4, 16.6. Calcd. for $C_{12}H_{22}NO_6P$: 46.90; H, 7.16 N, 4.56. Found: C, 46.85; H, 7.22; N, 4.51.

(S)-4-Isobutyl-*N*-diethylphosphoryl-acetyl-oxazolidin-2-one (3c)

Yield: 47%. Yellow oil. FT-IR (KBr, ν cm^{-1}): 1248 (P=O), 1005 (P-O), 1689.8 (C=O $_{amide}$), 1792.7 (C=O_{cycle}).^{31}P NMR (200 MHz, $CDCl_3$, δ ppm): 16.33. ^{1}H RMN (250 MHz, $CDCl_3$,δ ppm): 0.91-0.96 (2d, 6H, *J*=7,9, 8.4 Hz), 1.36(t, *J* = 7.2 Hz, 6H, (O-CH$_2$-C**H**$_3$)$_2$), 1.72 (m, 2H,CH*-C**H**$_2$-CH), 2.10 (m, 1H, C**H**-$(CH_3)_2$), 2.85 (d, $J_{H/P}$ = 3.2 Hz,2H, C**H**$_2$ -P), 4.19 (q, *J*= 7.1 Hz, 4H, (CH$_3$-C**H**$_2$) $_2$, 4.2 (m, 1H, C**H***), 4.4 (m, 2H,C**H**$_2$-CH*). ^{13}C NMR (, $CDCl_3$, δppm): 173.0, 152.1, 69.9, 61.5,49.2,44. 6,42. 1,25. 4,23. 6,22. 4,16.6. calcd.for $C_{13}H_{24}NO_6P$: C, 48.59; H, 7.47; N, 4.36. Found: C, 48.55; H, 7.42; N, 4.41.

(S)-4-benzyl-*N*-diethylphosphoryl-acetyl-oxazolidin-2-one (3d)
Yield: 52%. Yellow oilIR ($CDCl_3$, cm^{-1}): FT-IR (KBr, ν cm^{-1}): 1258 (P=O), 1008 (P-O), 1689.8 ($C=O_{amide}$), 1792.7 ($C=O_{cycle}$). ^{31}P NMR (200 MHz, $CDCl_3$, δ ppm): 16.22. ^{1}H NMR (250 MHz, $CDCl_3$, δ ppm): 1.36 (t, J = 7.2 Hz, 6H, (CH_3-CH_2)$_2$), 2.85 (d, $J_{H/P}$ = 3.2 Hz, 2H, CH_2 -P), 2.90 (dd, J= 13.2, 3.4 Hz, 1H, CH_2-Ph), 3.20 (dd, J = 13.6, 3.4 Hz, 1H, CH_2-Ph), 4.19 (q, J = 7.1 Hz, 4H, (CH_3-CH_2-O), 4.30 (m, 2H, CH_2-CH*), 4.58 (m, 1H, CH_2-CH*), 7.20-7.40 (m, 5H, H-Ar). ^{13}C NMR ($CDCl_3$, δ ppm): 174.0, 154.8, 137.2, 130.2, 128.9, 127.1, 67.1, 61.5, 52.8, 42.1, 38.9, 16.6. Calcd.for $C_{16}H_{22}NO_6P$: C, 54.08; H, 6.19; N, 3.94; Found: C, 54.05; H, 6.22; N, 3.98.

Cytotoxicity
The cellular growth constitutes the basic criteria that can make from an organism a model of survey[22]. Growth of microorganism can be quantified by increase of the size, the weight or the number. Nevertheless, there are several agents limiting this important criterion. *Paramecium sp* are unicellular ciliate, there is an efficient biological alternative model used to study environmental qualities and toxic effects of industrial, agricultural, in genetic, physiologic and morphologic study. The cytotoxicity of synthesized molecules (**2d and 3c**) was evaluated on the unicellular protiste. Toxic study was determined by the kinetics growth. Protistes sciliates were used in the evaluation of cytotoxic effects of xenobiotic they can be performed using different parameters.

Cell culture and treatment
The culture medium of paramecium was performed according to the method of Rouabhi et al[22]. Xenobiotics **2d** and **3c** was tested in aliquots 100 ml of culture (medium with and without aceton), three concentrations were chosen: 50, 100 and 200 µM.

Kinetics of Growth
Kinetics growth of paramecium is realised by spectrophotometry at λ = 600nm.

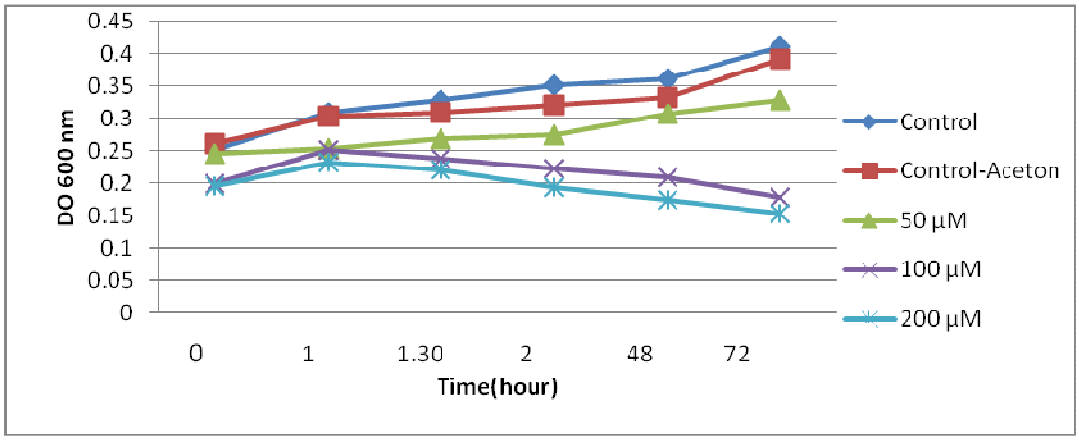


Fig.-1: Effect of molecule 2d at different concentrations on *paramecium sp* cell growth

The Fig.-1 represents the variation of Optical Density of molecule **2d** at 50 µM, 100 µM and 200 µM after 72h of treatment. Cells cultures exposed at 50 µM illustrate a similar cell growth to that control and control acetone OD = 0,307nm. Treated cell with 100 and 200 µM showed an important decrease of growth.
Treatment of *Paramecium sp* with molecule **3c** at concentrations 50, 100 and 200 µM, (Figure 2) showed a decrease in the growth of cells up 3 days at different concentrations. **3c** compound has an inhibitory effect on the population density growth of protistes.

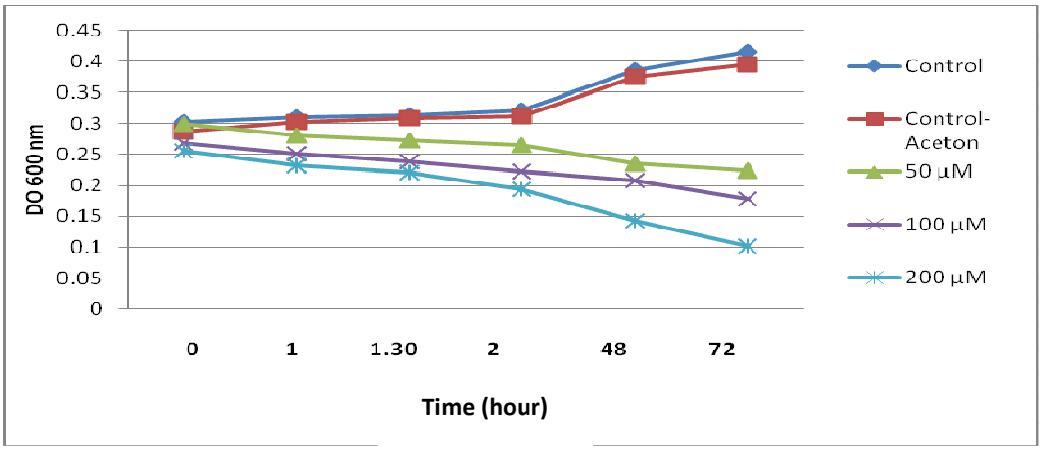


Fig.-2: Effect of molecule 3c on *paramecium sp*cell growth

CONCLUSION

In summary, four novel oxazolidinon-2-ones containing phosphonate moiety (**3a-3d**) have been synthesized in moderate yield. The synthesis has been performed easily starting from the precursor compounds, chloroacetyl-oxazolidin-2-one (**2a-2d**) and triethyl phosphite using Michaelis-Arbuzov reaction. The cytotoxicity of synthesized molecules (**2d and 3c**) showed promising results.The results suggest that the **2d** and **3c** molecules have a low cytotoxic effect on the studied cells, the high concentration of **2d, 3c** could constitute excellent potentially cytotoxic agents, in particularly in antitumor therapy. The study is extended to modified oxazolin-2-one-phosphonates, these compounds are currently being evaluated for antimicrobial activity, and the results of these investigations will be reported in due course.

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