Synthesis, spectral characterization and antimicrobial activity of α-hydroxyphosphonates

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ABSTRACT

A simple and efficient method for the synthesis of α-hydroxyphosphonates (3a-j) was accomplished by the reaction of aldehydes and dialkyl phosphites using 1,4-dimethyl piperazine as a catalyst under solvent-free condition at room temperature. Their chemical structures were characterized by IR, NMR (1H, 13C & 31P), Mass spectral and elemental analysis. All the titled compounds were screened for in vitro antimicrobial activity.

Keywords: α-Hydroxyphosphonates; phosphite; 1,4-dimethyl piperazine; antimicrobial activity;

INTRODUCTION

Phosphorus-carbon bond formation has attracted growing attention due to their novel application in organic synthesis and bio-organic chemistry. α-Functionalized phosphonates are valuable intermediates for the preparation of medicinal compounds and synthetic intermediates [1-3]. Among these compounds α-amino and α-hydroxyphosphonates are important class of compounds that exhibit a variety of interesting and useful applications [4]. In recent years, the preparation of α-hydroxyphosphonates has attracted significant attention, due to their potential biological activities such as enzyme inhibitors, anti-HIV, anti-oxidant, antimicrobial and antiviral properties [5-7]. In addition, they are useful intermediates in the synthesis of other phosphorous compounds [8-10]. These compounds may also be used as precursors for the synthesis of organophosphorus polymers possessing flame-resistant, corrosion-resistant, and ion-exchange properties [11-13]. Indeed, α-hydroxyphosphonates are also used as extractants for the recovery or separation of some metal ions [14]. Several different strategies for the synthesis of α-hydroxyphosphonates are known, the most common protocol is the reaction of aldehydes or ketones with dialkyl or trialkyl phosphites in the presence of acidic or basic catalysts. Meanwhile, tris(trimethylsilyl)phosphite was also used to synthesize α-hydroxyphosphonates but it requires elevated temperature under anhydrous reaction conditions [15-17]. However, many of these methods are associated with various drawbacks such as use of metal catalysts, tedious experimental procedures, unsatisfactory yields, long reaction times and usage of expensive, moisture sensitive catalysts. Hence, there is a need to develop a rapid and efficient protocol for the synthesis of α-hydroxyphosphonates.

In continuation of our ongoing research work on the development of useful synthetic methodologies [18-21], herein we report an efficient and practical method for the synthesis of α-hydroxyphosphonates using 1,4-dimethyl piperazine as a catalyst under solvent-free condition.
2.1. Experimental

All chemicals were procured from Sigma-Aldrich and used without further purification. The melting points (mp) were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. IR spectra were recorded in KBr pellets on a PerkinElmer 683 spectrophotometer. $^{1}$H, $^{13}$C and $^{31}$P NMR spectra were recorded on a Bruker instrument at 400 MHz in CDCl$_3$ using TMS as internal standard. $^{31}$P NMR (202.44 MHz) was taken in CDCl$_3$ using 85% H$_3$PO$_4$ as external standard with broadband $^1$H decoupling. Mass spectra were recorded on LCMS-2010A Shimadzu, Japan, spectrometer at University of Hyderabad, Hyderabad. Elemental analysis was performed using EA 1112 Thermo Finnigan, France, instrument at University of Hyderabad, Hyderabad, India.

2.2. General Procedure for the Synthesis of α-Hydroxyphosphonates

A mixture of aldehyde (1 mmol), diethyl phosphate (1.5 mmol) and 1,4-dimethyl piperazine (20 mol %) under neat conditions was stirred at room temperature. After completion of reaction as indicated by TLC, the contents were poured into water and extracted with ethyl acetate. The extract was dried with anhydrous Na$_2$SO$_4$ and evaporated under vacuum. The crude product was subsequently purified by column chromatography on 60-120 mesh silica gel using ethyl acetate - hexane (1:4) as eluent. This procedure was applied successfully for the preparation of 3a-f.

2.2.1. Dimethyl hydroxy (4-hydroxy-3-nitrophenyl) methylphosphonate (3a):

Solid, Yield: 89%, mp 91-93 °C. IR (KBr) ($\nu$): 7.82-7.22 (m, 3H, Ar-H), 5.55 (s, 1H, OH), 3.85 (s, 1H, OH), 4.46 (d, J = 10.5 Hz, 1H, PCH), 3.62-3.45 (m, 3H, POCH$_2$), 134.5 (C-6), 129.5 (C-1), 125.1 (C-2), 120.2 (C-5), 70.3 (d, 1H, OH, POC$_3$), 53.7 (d, J = 6.5 Hz, POCH$_2$). $^{13}$C NMR (CDCl$_3$, 100.57 MHz): 152.5 (C-4), 137.0 (C-3), 134.5 (C-6), 129.5 (C-1), 125.1 (C-2), 120.2 (C-5), 70.3 (d, J = 157.0 Hz, P-CH$_3$), 53.7 (d, J = 6.0 Hz, POCH$_3$), $^{31}$P NMR (CDCl$_3$, 161.9 MHz): $\delta$ 22.82; ESI-MS: m/z 278 (M+H)$^+$; Anal. calcd. for C$_9$H$_{12}$NO$_2$P: C, 39.00; H, 4.36; N, 5.05; Found: C, 38.58; H, 4.20, N, 5.01;

2.2.2. Diethyl hydroxy (4-hydroxy-3-nitrophenyl) methylphosphonate (3b):

Solid, Yield: 94%, mp 112-114 °C. IR (KBr) ($\nu$_max cm$^{-1}$): 3272 (brs, OH), 1243 (P=O), 1040 (P-O-C); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 7.46-7.23 (m, 3H, Ar-H), 5.15 (s, 1H, OH), 4.79 (d, J = 10.9 Hz, 1H, PCH), 4.32-4.17 (m, 2H, POCH$_2$), 4.12-3.99 (m, 2H, POCH$_2$), 3.52 (s, 1H, OH), 1.35-1.24 (m, 3H, CH$_3$), 1.20-1.12 (m, 3H, CH$_3$); $^{13}$C NMR (CDCl$_3$, 100.57 MHz): 152.2 (C-4), 137.5 (C-3), 133.2 (C-6), 128.3 (C-1), 124.2 (C-2), 121.5 (C-5), 71.2 (d, J = 158.2 Hz, P-CH$_3$), 53.5 (d, J = 6.4 Hz, POCH$_2$), 16.9 (d, J = 5.8 Hz, CH$_3$), 16.4 (d, J = 5.8 Hz, CH$_3$); $^{31}$P NMR (CDCl$_3$, 161.9 MHz): $\delta$ 20.8; ESI-MS: m/z 306 (M+H)$^+$; Anal. calcd. for C$_{14}$H$_{18}$NO$_3$P: C, 43.29; H, 5.28; N, 4.59; Found: C, 43.18; H, 5.21; N, 4.48;

2.2.3. Dibutyl hydroxy (4-hydroxy-3-nitrophenyl) methylphosphonate (3c):

Solid, Yield: 84%, mp 87-89 °C. IR (KBr) ($\nu$_max cm$^{-1}$): 3254 (brs, OH), 1243 (P=O), 1035 (P-O-C); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 7.48-7.21 (m, 3H, Ar-H), 5.46 (s, 1H, OH), 4.75 (d, J = 11.2 Hz, 1H, PCH), 4.32-4.17 (m, 2H, POCH$_2$), 4.12-3.99 (m, 2H, POCH$_2$), 3.46 (s, 1H, OH), 1.71-1.55 (m, 4H, CH$_2$), 1.50-1.37 (m, 4H, CH$_2$), 1.32-1.21 (m, 3H, CH$_3$), 1.21-1.13 (m, 3H, CH$_3$); $^{13}$C NMR (CDCl$_3$, 100.57 MHz): 153.5 (C-4), 139.7 (C-3), 131.2 (C-6), 129.5 (C-1), 126.8 (C-2), 120.8 (C-5), 72.8 (d, J = 154.4 Hz P-CH$_3$), 54.8 (d, J = 6.5 Hz, POCH$_2$), 54.2 (d, J = 6.2 Hz, POCH$_2$), 30.2 (CH$_3$), 30.1 (CH$_3$), 18.2 (CH$_3$), 18.0 (CH$_3$), 16.8 (d, J = 5.8 Hz, CH$_3$), 16.2 (d, J = 5.8 Hz, CH$_3$); $^{31}$P NMR (CDCl$_3$, 161.9 MHz): $\delta$ 22.4; ESI-MS: m/z 362 (M+H)$^+$; Anal. calcd. for C$_{16}$H$_{22}$NO$_4$P: C, 49.86; H, 6.69; N, 3.88; Found: C, 49.78; H, 6.62; N, 3.79;

2.2.4. Diphenyl hydroxy (4-hydroxy-3-nitrophenyl) methylphosphonate (3d):

Solid, Yield: 84%, mp 87-89 °C. IR (KBr) ($\nu$_max cm$^{-1}$): 3332 (brs, OH), 1249 (P=O), 1038 (P-O-C); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 8.46-7.21 (m, 13H, Ar-H), 5.26 (s, 1H, OH), 4.61 (d, J = 10.8 Hz, 1H, PCH), 4.32 (s, 1H, OH); $^{13}$C NMR (CDCl$_3$, 100.57 MHz): 155.9 (C-4), 150.2 (C-1'), 149.8 (C-1), 138.5 (C-3), 134.2 (C-6), 131.7 (C-3'), 130.9 (C-3'' & C-5'), 130.1 (C-1'), 126.8 (C-2), 122.4 (C-4'), 122.1 (C-4''), 120.9 (C-5), 120.4 (C-2' & C-6'), 120.0 (C-2'' & C-6''), 75.8 (d, J = 152.8 Hz P-CH$_3$); $^{31}$P NMR (CDCl$_3$, 161.9 MHz): $\delta$ 22.4; ESI-MS: m/z 402 (M+H)$^+$; Anal. calcd. for C$_{20}$H$_{26}$NO$_5$P: C, 56.87; H, 4.02; N, 3.49; Found: C, 56.81; H, 3.95; N, 3.37;

2.2.5. Dibenzyldimethylhydroxy (4-hydroxy-3-nitrophenyl) methylphosphonate (3e):

Solid, Yield: 88%, mp 103-105 °C. IR (KBr) ($\nu$_max cm$^{-1}$): 3235 (brs, OH), 1252 (P=O), 1028 (P-O-C); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 8.44-7.18 (m, 13H, Ar-H), 5.34 (s, 2H, Ph-CH$_2$), 5.30 (s, 2H, Ph-CH$_2$), 5.28 (s, 1H, OH), 4.49
2.2.6. Dimethyl (4-chloro-3-nitrophenyl)(hydroxy)methylyphosphonate (3f):
Solid, Yield: 86 %, mp 75-77 ºC. IR (KBr) (v_max cm⁻¹): 3321 (brs, OH), 1238 (P=O), 1032 (P-O-C); ¹H NMR (CDCl₃, 400 MHz) δ: 7.37-7.24 (m, 3H, Ar-H), 1.42 (d, J = 10.2 Hz, 1H, PCH), 3.69 (s, 1H, OH), 3.60-3.42 (m, 3H, POCH₂) 3.40-3.22 (m, 3H, POCH₂); ¹³C NMR (CDCl₃, 100.57 MHz) δ: 147.4 (C-3), 134.2 (C-6), 133.8 (C-1), 130.2 (C-5), 126.4 (C-4), 124.8 (C-2), 74.2 (d, J = 152.4 Hz, P-CH), 53.8 (d, J = 6.4 Hz, POCH₂), 53.5 (d, J = 6.2 Hz, POCH₂); ³¹P NMR (CDCl₃, 161.19 MHz): δ 21.23; ESI-MS: m/z 296 (M+H)⁺; Anal. calcd. for C₅H₁₁NO₃P: C, 36.57; H, 3.75; N, 4.74; Found: C, 36.56; H, 3.68; N, 4.65.

2.2.7. Diethyl (4-chloro-3-nitrophenyl)(hydroxy)methylyphosphonate (3g):
Solid, Yield: 92 %, mp 118-120 ºC. IR (KBr) (v_max cm⁻¹): 3330 (brs, OH), 1232 (P=O), 1048 (P-O-C); ¹H NMR (CDCl₃, 400 MHz) δ: 7.49-7.18 (m, 3H, Ar-H), 4.72 (d, J = 10.2 Hz, 1H, PCH), 4.28-4.20 (m, 2H, POCH₂), 1.36-1.15 (m, 3H, CH₃), 1.19-1.12 (m, 3H, CH₃); ¹³C NMR (CDCl₃, 100.57 MHz) δ: 147.4 (C-3), 134.2 (C-6), 133.8 (C-1), 130.2 (C-5), 126.4 (C-4), 124.8 (C-2), 74.2 (d, J = 152.4 Hz, P-CH), 53.3 (d, J = 6.4 Hz, POCH₂), 16.8 (d, J = 6.2 Hz, CH₃), 16.4 (d, J = 6.0 Hz, CH₃); ³¹P NMR (CDCl₃, 161.19 MHz): δ 21.6; ESI-MS: m/z 324 (M+H)⁺; Anal. calcd. for C₅H₁₁NO₃P: C, 40.82; H, 4.67; N, 4.33; Found: C, 40.72; H, 4.61; N, 4.24;

2.2.8. Dibutyl (4-chloro-3-nitrophenyl)(hydroxy)methylyphosphonate (3h):
Solid, Yield: 92%, mp 95-97 ºC. IR (KBr) (v_max cm⁻¹): 3252 (brs, OH), 1228 (P=O), 1032 (P-O-C); ¹H NMR (CDCl₃, 400 MHz) δ: 7.52-7.20 (m, 3H, Ar-H), 4.72 (d, J = 11.4 Hz, 1H, PCH), 4.30-4.21 (m, 2H, POCH₂), 4.18-4.08 (m, 2H, POCH₂), 3.40 (s, 1H, OH), 1.70-1.58 (m, 4H, CH₂), 1.52-1.37 (m, 4H, CH₂), 1.34-1.26 (m, 3H, CH₃), 1.22-1.16 (m, 3H, CH₃); ¹³C NMR (CDCl₃, 100.57MHz): δ 147.8 (C-3), 135.2 (C-6), 138.4 (C-1), 132.2 (C-5), 126.9 (C-4), 124.9 (C-2), 76.6 (d, J = 150.8 Hz, P-CH), 56.8 (d, J = 6.8 Hz, POCH₂), 56.2 (d, J = 6.4 Hz, POCH₂), 32.2 (CH₂), 31.8 (CH₂), 18.4 (CH₂), 18.2 (CH₂), 16.6 (d, J = 6.0 Hz, CH₃), 16.3 (d, J = 5.8 Hz, CH₃); ³¹P NMR (CDCl₃, 161.19 MHz): δ 20.8; ESI-MS: m/z 380 (M+H)⁺; Anal. calcd. for C₁₅H₂₉NO₃P: C, 47.44; H, 6.10; N, 3.69; Found: C, 47.38; H, 6.02; N, 3.60;

2.2.9. Diphenyl hydroxy(4-hydroxy-3-nitrophenyl)methylyphosphonate (3i):
Solid, Yield: 88%, mp 83-85 ºC. IR (KBr) (v_max cm⁻¹): 3342 (brs, OH), 1240 (P=O), 1030 (P-O-C); ¹H NMR (CDCl₃, 400 MHz) δ: 8.32-7.68 (m, 13H, Ar-H), 4.62 (d, J = 10.2 Hz, 1H, PCH), 4.38 (s, 1H, OH); ¹³C NMR (CDCl₃, 100.57 MHz) δ: 150.2 (C-1'), 150.0 (C-1''), 147.2 (C-3), 134.8 (C-1), 134.2 (C-6), 132.2 (C-5), 131.8 (C-3' & C-5'), 130.8 (C-3'' & C-5''), 126.8 (C-4), 124.8 (C-2), 123.4 (C-4'), 123.0 (C-5'), 120.4 (C-2' & C-6'), 120.1 (C-2'' & C-6''), 76.6 (d, J = 150.2 Hz P-CH). ³¹P NMR (CDCl₃, 161.19 MHz): δ 21.8; ESI-MS: m/z 420 (M+H)⁺; Anal. calcd. for C₁₀H₁₃NO₃P: C, 54.37; H, 3.60; N, 3.34; Found: C, 54.30; H, 3.52; N, 3.24;

2.2.10. Dibenzhydryl hydroxy(4-hydroxy-3-nitrophenyl)methylyphosphonate (3j):
Solid, Yield: 88 %, mp 125-127 ºC. IR (KBr) (v_max cm⁻¹): 3232 (brs, OH), 1242 (P=O), 1030 (P-O-C); ¹H NMR (CDCl₃, 400 MHz) δ: 8.25-7.24 (m, 13H, Ar-H), 5.32 (s, 2H, Ph-CH₂), 5.30 (s, 2H, Ph-CH₂), 4.42 (d, J = 10.2 Hz, 1H, PCH), 4.32 (s, 1H, OH); ¹³C NMR (CDCl₃, 100.57 MHz) δ: 148.5 (C-3), 135.2 (C-1'), 135.0 (C-1''), 134.2 (C-1), 134.0 (C-6), 132.4 (C-5), 128.4 (C-3' & C-5'), 128.1 (C-3'' & C-5''), 127.4 (C-2' & C-6'), 127.2 (C-2'' & C-6''), 126.8 (C-4), 126.4 (C-2'), 126.2 (C-4'), 124.2 (C-2), 74.6 (d, J = 152.8 Hz P-CH) 64.2 (d, J = 6.2 Hz, POCH₂), 64.0 (d, J = 6.2 Hz, POCH₂); ³¹P NMR (CDCl₃, 161.19 MHz): δ 20.2; ESI-MS: m/z 448 (M+H)⁺; Anal. calcd. for C₂₉H₂₆NO₃P: C, 56.32; H, 4.28; N, 3.13; Found: C, 56.24; H, 4.20; N, 3.04;

2.3. Antimicrobial Activity
Antibacterial activity [22] of 3a-j was tested against the growth of *Staphylococcus aureus* (ATCC 25923) (gram ++ve) and *Escherichia coli* (ATCC 25922) (gram –ve) by disc diffusion method at various concentrations (50, 100 ppm) and results were summarized in Table 1. Penicillin was used as the reference compound. The compounds 3a-j showed moderate activity against Staphylococcus aureus and Escherichia coli. The highlight is that the three compounds 3e, 3g and 3j were more effective.

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Table 1: Antibacterial activity of compounds 3a-j (µg/mL)

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<th>Compound</th>
<th>Zone of inhibition (%)</th>
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Penicillin* 8 12 7 10

They were also screened for antifungal activity [22] against Aspergillus niger (ATCC 16404) and Helminthosporium oryzae (ATCC 11000) species along with the standard fungicide Griseofulvin by the disc diffusion method at two different concentrations (50, 100 ppm) and results were summarized in Table 2. It is gratifying to observe that the majority of the compounds (3a-j) exhibited higher antifungal activity against both tested fungal strains when compared with that of griseofulvin. The significant results are that 3e and 3j exhibited higher activities than the standard griseofulvin against both the fungi.

Table 2: Antifungal activity of compounds 3a-j (µg/mL)

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Griseofulvin* 7 12 9 12

RESULTS AND DISCUSSION

Initially we have synthesized α-hydroxyphosphonates (3a-j) by the reaction of aldehydes (1a,b) and dialkylphosphites (2a-e) in the presence of 1,4-dimethyl piperazine (20 mol %) as catalyst under solvent-free conditions at room temperature (Scheme 1).

Scheme 1: Synthesis of α-Hydroxyphosphonates (3a-j)
In initial endeavour we carried out the reaction of 4-hydroxy-3-nitrobenzaldehyde and dimethyl phosphite in the absence of 1,4-dimethyl piperazine. It was observed that there is no formation of product even after 6 hours of reaction time. After that by the addition of 1,4-dimethyl piperazine to the reaction mixture in catalytic amount (20 mol %) the reaction proceeded smoothly and afforded 88 % yield of the product within 3 h of reaction time. Which indicates that 1,4-dimethyl piperazine is necessary for this transformation. After that we extended this process with aldehydes and various dialkylphosphite to synthesize 3a-j. It was observed that all the reactions were successfully completed within 3-4 hours and gave the corresponding α-hydroxyphosphate in 84–93% yields. The chemical structures of all the products were fully characterized by IR, 1H, 13C\textsuperscript{31}P NMR and mass spectral data. The antimicrobial activity of the title compounds was evaluated by the standard methods and the results were summarized in Table 1 & 2.

CONCLUSION

In conclusion, 1,4-dimethyl piperazinewas found to be an efficient catalyst for the synthesis of α-hydroxyphosphonates by reaction of aldehydes and dialkylphosphite under solvent-free condition at room temperature. The main advantages of the present synthetic protocol are mild, solvent-free conditions and easy work-up procedure. It is expected that the present methodology will find application in organic synthesis. All the titled compounds were screened for antimicrobial activity and some of the compounds (3a-j) were exhibit high activity compared to reference standard.

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