Bull. Chem. Soc. Ethiop. **2009**, 23(1), 69-75. ISSN 1011-3924
Printed in Ethiopia

SYNTHESIS, CHARACTERIZATION AND BIO-ACTIVITY OF SOME NEW α-AMINOPHOSPHONATES

A. Balakrishna[1], C. Suresh Reddy[1*], S.K. Naik[2], M. Manjunath[3] and C. Naga Raju[1]

[1]Department of Chemistry, S.V. University, Tirupati-517 502, India
[2]Bio-organic Division, Bhabha Atomic Research Centre, Mumbai-400 085, India
[3]Department of Biochemistry, S.V. University, Tirupati-517 502, India

(Received June 19, 2008; revised December 10, 2008)

ABSTRACT. A convenient and efficient one-pot reaction has been employed for the synthesis of new α-aminophosphonates **4a-4k** *via* Kabachnik-Fields reaction in 65-82 % yields. In the procedure developed, equimolar quantities of 2-amino-4-methylphenol **1**, various aromatic aldehydes **2a-2k** and dimethylphosphite **3** in dry toluene were reacted under reflux for 4-6 h. The products were characterized by IR, ^{1}H, ^{13}C and ^{31}P NMR spectra and elemental analysis. All the synthesized compounds were screened for in vitro antibacterial activity (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*) and antifungal activity (*Candida albicans*, *Aspergillus niger*). **4a** showed potent activity with lowest MIC value.

KEY WORDS: α-Aminophosphonate, Kabachnik-fields reaction, Antibacterial and antifungal activities

INTRODUCTION

Organophosphorus compounds have wide range of applications in the industrial, agricultural and medicinal chemistry owing to their unique physicochemical and biological properties. Their utility as reagents and potential synthons in organic synthesis is gaining increased attention [1].

Synthesis of α-aminophosphonates, structural analogues of natural amino acids, is receiving great attention due to their applications in agriculture as plant growth regulators, herbicides [2] and virucides [3] and in medicine as anti-cancer agents [4], enzyme inhibitors [5], peptide mimetics [6], antibiotics and pharmacological agents [7]. As a result, a variety of synthetic approaches [8] have been developed for the synthesis of α-aminophosphonates. Of them, Kabachnik-Fields three-component reaction, in which an aldehyde, an amine and a dialkyl phosphite are reacted in one-pot set-up, is an important one. In some reports, this reaction was carried out in straight-forward one-pot procedures without any catalysts [9, 10]. While, in most cases it was performed using catalysts, such as $LiClO_4$ [11, 12], $TaCl_5$-SiO_2 [13], $InCl_3$ [14], lanthanide-triflate [15] and CF_3COOH [16]. The key step in this synthesis is the nucleophilic addition of an amine to carbonyl compound followed by addition of a dialkyl (or) diarylphosphite to the resulting imine. Formation of hydroxy phosphonates and product of rearrangement frequently accompany this reaction.

In view of greater applicability of α-aminophosphonates, an easy one-pot synthetic procedure for them by Kabachnik-Fields reaction under mild conditions without catalyst has been developed.

EXPERIMENTAL

General

The chemicals procured were of commercial quality or chemically pure. All solvents were dried, deoxygenated and redistilled before use. The IR spectra (KBr pellets and Nujol mulls) were

*Corresponding author. E-mail: csrsvu@gmail.com

recorded on a Perkin-Elmer 283 unit. ^{1}H and ^{13}C spectra were recorded on a Bruker AMX 400 MHz spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as internal standard. ^{31}P NMR spectra were measured using 85 % H_3PO_4 as external reference. Elemental analyses were recorded on a Carlo Erba 1108 Elemental Analyser, Central Drug Research Institute, Lucknow, India. Mass spectra were recorded on Jeol 5 x 102 DA/600 mass–spectrometer using argon/xenon (6 keV, 10 mA) as the fast atom bombardment (FAB) gas. Melting points were determined in an open capillary tube on Mel-temp apparatus, Tempo instruments, India and were uncorrected. The following abbreviations were used while presenting the NMR data s = singlet, d = doublet, t = triplet and m = multiplet.

General procedure

*[5-Methyl-2-hydroxyphenyl amino)-(4-fluorophenyl)-methyl]-phosphonic acid dimethyl ester (**4a**).* A mixture of 2-amino-4-methylphenol **1** (0.72 g, 0.005 mol) and 4-fluorobenzaldehyde **2a** (0.005 mol) was stirred in anhydrous toluene (15 mL) at room temperature for 1 h. Dimethylphosphite **3** (0.7 mL, 0.005 mol) in anhydrous toluene (15 mL) was added dropwise. Stirring was continued at room temperature for another 30 min, after which the mixture was heated under reflux for 4-6 h. The reaction was monitored by TLC on silica gel using petroleum ether and ethyl acetate (1:2 v/v). After completion of the reaction, the solvent was removed by rotaevaporator and the resulting residue was purified by column chromatography on silica gel (100-200 mesh, ethyl acetate/hexane) as eluent to afford pure α-amino-phosphonate (**4a**). The other compounds **4b** to **4k** were prepared employing a procedure similar to that described for compound **4a**.

Yield: 1.38 g, 82 %, m.p.: 270-272 °C. IR (KBr): ν (cm^{-1}) 3350 (-N-H); 1245 (-P=O); 752 (-P-$C_{aliphatic}$); 3422 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.37 (s, 1H, Ar-OH), 6.44-7.51 (m, 7H, aromatic); 5.18 dd ($^{2}J_{PH}$ = 24.1 Hz, $^{3}J_{HH}$ = 9.9 Hz, (P-C-H), 5.00, (t, $^{3}J_{PH}$ = 8.6 Hz, $^{3}J_{HH}$ = 9.6 Hz, PCNH), 3.45 (d, J_{POCH} = 10.4 Hz, P-OCH$_3$), 3.65 (d, J_{POCH} = 10.4 Hz, P-OCH$_3$), 2.34 (s, 3H, Ar-CH$_3$). ^{31}P NMR (DMSO-d_6): ppm 27.52. ^{13}C NMR data: ppm 129.6 (C-1); 142.4 (C-2); 113.6 (C-3); 117.8 (C-4); 127.8 (C-5); 112.6 (C-6); 51.7 (C-7); 132.7 (C-1′); 134.4 (C-2′); 115.0 (C-3′); 162.7 (C-4′); 115.0 (C-5′); 134.3 (C-6′); 51.7 (s, P-OCH$_3$), 52.5 (d, $^{1}J_{PC}$ = 149.9 Hz, P-C), 20.68 (Ar-CH$_3$). FAB MS: *m/z* (%) 338 (25, M$^{+\bullet}$), 339 (80), 307 (11.1), 291 (22.2), 244 (50), 230 (100), 228 (69), 154 (50), 136 (47.2), 134 (41.6), 107 (41.6). Anal. calcd. for $C_{16}H_{19}NO_4PF$: C, 56.63; H, 5.64; N, 4.12. Found: C, 56.52; H, 5.59; N, 4.08.

*[5-Methyl-2-hydroxyphenyl amino)-(m-nitrophenyl)-methyl]-phosphonic acid dimethyl ester (**4b**).* Yield: 1.42 g, 78 %; m.p.: 180-182 °C. IR (KBr): ν (cm^{-1}) 3345 (-N-H); 1206 (-P=O); 761 (-P-$C_{aliphatic}$); 3418 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.41 (s, 1H, Ar-OH), 6.24-8.31 (m, 7H, aromatic); 5.48 (dd, 1H, $^{2}J_{PH}$ = 24.0 Hz, $^{3}J_{HH}$ = 9.8 Hz, PCH), 5.11 (t, 1H, J_{PH} = 9.2 Hz, $^{3}J_{HH}$ = 9.2 Hz, (PCNH)), 3.53 (d, J_{POCH} = 10.8 Hz, P-OCH$_3$), 3.70 (d, J_{POCH} = 10.6 Hz, P-OCH$_3$), 2.0 (s, 3H, Ar-CH$_3$). ^{31}P NMR (DMSO-d_6), δ: 26.60 ppm. ^{13}C NMR data: ppm 134.3 (C-1); 142.4 (C-2); 113.8 (C-3); 118.2 (C-4); 129.8 (C-5); 112.7 (C-6); 51.7 (C-7); 139.4 (C-1′); 122.2 (C-2′); 147.7 (C-3′); 122.6 (C-4′); 127.9 (C-5′); 134.0 (C-6′); 51.7 (s, P-O-CH$_3$), 52.40 (d, $^{1}J_{PC}$ = 149 Hz, P-C), 20.66 (Ar-CH$_3$). FAB MS : *m/z* (%): 366 (23, M$^{+\bullet}$), 291 (42.8), 289 (34), 257 (85.7), 154 (100), 136 (65), 107 (34). Anal. calcd. for $C_{16}H_{19}N_2O_6P$: C, 52.47; H, 5.22; N, 7.64. Found: C, 52.35; H, 5.17; N, 7.59.

*[5-Methyl-2-hydroxyphenyl amino)-(4-diethyl amino phenyl)-methyl]-phosphonic acid dimethyl ester (**4c**).* Yield: 1.27 g, 65 %; m.p.: 280-282 °C. IR (KBr): ν (cm^{-1}) 3401 (-N-H); 1202 (-P=O); 751 (-P-$C_{aliphatic}$); 3406 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.45 (s, 1H, Ar-OH), 6.33-7.95 (m, 7H, aromatic); 4.85 (dd, $^{2}J_{PH}$ = 19.4 Hz, $^{3}J_{HH}$ = 9.2 Hz, P-C-H), 4.30 (t, 1H, $^{3}J_{PH}$ = 9.6

Hz, $^{3}J_{HH}$ = 9.1 Hz, PC<u>NH</u>), 3.48 (d, J_{POCH} = 10.2 Hz, P-O<u>CH</u>$_3$), 3.64 (d, J_{POCH} = 10.4 Hz, P-O<u>CH</u>$_3$), 2.08 (s, 3H, Ar-CH_3), 3.22-3.40 (m, 4H, N-CH_2), 0.98-1.22 (m, 6H, N-CH_3). ^{31}P NMR (DMSO-d_6): ppm 27.41. ^{13}C NMR data: ppm 130.1 (C-1); 142.3 (C-2); 114.7 (C-3); 121.9 (C-4); 133.5 (C-5); 113.6 (C-6); 51.8 (C-7); 133.6 (C-1′); 128.9 (C-2′); 111.9 (C-3′); 146.9 (C-4′); 111.2 (C-5′); 128.6 (C-6′); 51.8 (s, P-OCH_3), 52.4 (d, $^{1}J_{PC}$ = 121.4 Hz, P-C), 42.7 (N-<u>CH_2</u>-CH_3), 12.39 (N-CH_2 CH_3)$_2$, 19.04 (Ar-CH_3). Anal. calcd. for $C_{20}H_{29}N_2O_4P$: C, 61.22; H, 7.44; N, 7.19. Found: C, 61.12; H, 7.39; N, 7.12.

[5-Methyl-2-hydroxyphenyl amino)-(4-isopropylphenyl)-methyl]-phosphonic acid dimethyl ester ***(4d)***. Yield: 1.17 g, 65 %; m.p.: 182-184 °C. IR (KBr): ν (cm^{-1}) 3386 (-N-H); 1209 (-P=O); 754 (-P-$C_{aliphatic}$); 3396 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.41 (s, 1H, Ar-OH), 6.43-7.48 (m, 7H, aromatic); 4.97 (dd, 1H, $^{2}J_{PH}$ = 20.8 Hz, $^{3}J_{HH}$ = 9.8 Hz, P-C-<u>H</u>), 4.77 (t, 1H, $^{3}J_{PH}$ = 9.2 Hz, $^{3}J_{HH}$ = 9.1 Hz, PC<u>NH</u>), 3.46 (d, J_{POCH} = 9.5 Hz, P-O<u>CH</u>$_3$), 3.52 (d, J_{POCH} = 8.2 Hz, P-O<u>CH</u>$_3$), 2.18 (s, 3H, Ar-CH_3), 2.62-2.68(m,1H, (C<u>H</u>)(CH_3)$_2$), 1.17 (d, 3H, J = 7.2 Hz (CH)(C<u>H</u>$_3$)$_2$). ^{31}P NMR (DMSO-d_6): ppm 18.59. Anal. calcd. for $C_{19}H_{26}NO_4P$: C, 62.79; H, 7.21; N, 3.85. Found: C, 62.69; H, 7.16; N, 3.80.

[5-Methyl-2-hydroxyphenyl amino)-(4-tert.butylphenyl)-methyl]-phosphonic acid dimethyl ester ***(4e)***. Yield: 1.26 g, 67 %; m.p.: 274-276 °C. IR (KBr): ν (cm^{-1}) 3446 (-N-H); 1215 (-P=O); 750 (-P-$C_{aliphatic}$); 3398 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.24 (s, 1H, Ar-OH), 6.21-7.48 (m, 7H, aromatic); 4.72 (dd, 1H, $^{2}J_{PH}$ = 21.0 Hz, $^{3}J_{HH}$ = 9.1 Hz, P-C-<u>H</u>), 4.61 (t, 1H, $^{3}J_{PH}$ = 10.4 Hz, $^{3}J_{HH}$ = 10.1 Hz, PC<u>NH</u>), 3.49 (d, J_{POCH} = 11.5 Hz, P-O<u>CH</u>$_3$), 3.57 (d, J_{POCH} = 11.8 Hz, P-O<u>CH</u>$_3$), 2.37 (s, 3H, Ar-CH_3), 2.06 (s, 9H, 4C $(CH_3)_3$). ^{31}P NMR (DMSO-d_6): ppm 18.76. Anal. calcd. for $C_{20}H_{28}NO_4P$: C, 63.64; H, 7.47; N, 3.71. Found: C, 63.55; H, 7.43; N, 3.65.

[5-Methyl-2-hydroxyphenyl amino)-(4-hydroxyphenyl)-methyl]-phosphonic acid dimethyl ester ***(4f)***. Yield: 1.12 g, 70 %; m.p.: 184-185 °C. IR (KBr): ν (cm^{-1}) 3384 (-N-H); 1215 (-P=O); 775 (-P-$C_{aliphatic}$); 3432 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.20 (s, 1H, Ar-OH), 6.06-6.89 (m, 7H, aromatic); 5.09 (dd, 1H, $^{2}J_{PH}$ = 23.0 Hz, $^{3}J_{HH}$ = 8.8 Hz, P<u>CH</u>), 4.92 (t, 1H, $^{3}J_{PH}$ = 10.6 Hz, $^{3}J_{HH}$ = 9.9 Hz, PC<u>NH</u>), 3.48 (d, J_{POCH} = 10.6 Hz, P-O<u>CH</u>$_3$), 3.65 (d, J_{POCH} = 9.6 Hz, P-O<u>CH</u>$_3$), 2.35 (s, 3H, Ar-CH_3), 10.08 (s, 1H, 4R′-OH). ^{31}P NMR (DMSO-d_6): ppm 27.08. Anal. calcd. for $C_{16}H_{20}NO_5P$: C, 56.98; H, 5.97; N, 4.15. Found: C, 56.87; H, 5.94; N, 4.09.

[5-Methyl-2-hydroxyphenyl amino)-(4-methylphenyl)-methyl]-phosphonic acid dimethyl ester ***(4g)***. Yield: 1.23 g, 74 %; m.p.: 275-278 °C. IR (KBr): ν (cm^{-1}) 3353 (-N-H); 1212 (-P=O); 771 (-P-$C_{aliphatic}$); 3404 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.1 (s, 1H, Ar-OH), 6.11-6.89 (m, 7H, aromatic); 4.99 (dd, 1H, $^{2}J_{PH}$ = 18.3 Hz, $^{3}J_{HH}$ = 9.2 Hz, P-C-<u>H</u>), 4.93 (t, 1H, $^{3}J_{PH}$ = 10.8 Hz, $^{3}J_{HH}$ = 9.7 Hz, PC<u>NH</u>), 3.53 (d, J_{POCH} = 10.9 Hz, P-O<u>CH</u>$_3$), 3.69 (d, J_{POCH} = 10.4 Hz, P-O<u>CH</u>$_3$), 2.38 (s, 3H, Ar-CH_3), 2.38 (s, 3H, 4R′-CH_3). ^{31}P NMR (DMSO-d_6): ppm 27.98. Anal. calcd. for $C_{17}H_{22}NO_4P$: C, 60.90; H, 6.60; N, 4.17. Found: C, 60.79; H, 6.56; N, 4.12.

[5-Methyl-2-hydroxyphenylamino)-(4-methoxyphenyl)-methyl]-phosphonic acid dimethyl ester ***(4h)***. Yield: 1.26 g, 72 %; m.p.: 125-127 °C. IR (KBr): ν (cm^{-1}) 3343 (-N-H); 1202 (-P=O); 761 (-P-$C_{aliphatic}$); 3412 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.81 (s, 1H, Ar-OH), 6.68-7.81 (m, 7H, aromatic); 5.52 (dd, 1H, $^{2}J_{PH}$= 24.4 Hz, $^{3}J_{HH}$ = 10.0 Hz, P-C-<u>H</u>), 5.44 (t, 1H, $^{3}J_{PH}$ = 10.9 Hz, $^{3}J_{HH}$ = 9.9 Hz, PC<u>NH</u>), 3.88 (d, J_{POCH} = 10.6 Hz, P-O<u>CH</u>$_3$), 4.13 (d, J_{POCH} = 10.6 Hz, P-O<u>CH</u>$_3$), 2.49 (s, 3H, Ar-CH_3), 3.76 (s, 3H, Ar-OCH_3). ^{31}P NMR (DMSO-d_6) : 28.04 ppm. ^{13}C NMR data: ppm 130.6 (C-1); 138.7 (C-2); 113.7 (C-3); 117.7 (C-4); 127.9 (C-5); 112.7 (C-6); 55.5 (C-7); 142.4 (C-1′); 134.7 (C-2′); 128.9 (C-3′); 178.9 (C-4′); 128.2 (C-5′); 134.6 (C-6′); 51.9 (s, P-OCH_3), 52.6 (d, $^{1}J_{PC}$ = 146.9 Hz, P-C), 20.77 (Ar-CH_3). FAB MS: *m/z* (%): 351 (25, $M^{+\bullet}$), 325 (36), 323

(20), 242 (100), 136 (55.5), 109 (52.7). Anal. calcd. for $C_{17}H_{22}NO_5P$: C, 58.13; H, 6.30; N, 3.98. Found: C, 58.03; H, 6.25; N, 3.92.

*[5-Methyl-2-hydroxyphenyl amino)-(4-chlorophenyl)-methyl]-phosphonic acid dimethyl ester (**4i**)*. Yield: 1.40 g, 81 %; m.p.: 218-220 °C. IR (KBr): ν (cm^{-1}) 3389 (-N-H); 1247 (-P=O); 755 (-P-$C_{aliphatic}$); 3410 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.21 (s, 1H, Ar-OH), 6.05-7.68 (m, 7H, aromatic); 5.29 (dd, $^2J_{PH}$ = 17.3 Hz, $^3J_{HH}$ = 10.0 Hz, P-C-H), 5.22 (t, 1H, $^3J_{PH}$ = 9.7 Hz, $^3J_{HH}$ = 9.0 Hz, PCNH), 3.49 (d, J_{POCH} = 10.6 Hz, P-OCH_3), 3.65 (d, J_{POCH} = 10.4 Hz, P-OCH_3), 2.57 (s, 1H, Ar-CH_3). ^{31}P NMR (DMSO-d_6): ppm 27.04. ^{13}C NMR data: ppm 130.0 (C-1); 139.4 (C-2); 113.8 (C-3); 118.0 (C-4); 132.9 (C-5); 112.9 (C-6); 52.0 (C-7); 142.3 (C-1′); 127.7 (C-2′); 126.6 (C-3′); 134.3 (C-4′); 126.6 (C-5′); 127.9 (C-6′); 52.0 (s, P-OCH_3), 52.6 (d, $^1J_{PC}$ = 131.8 Hz, P-C), 20.1 (Ar-CH_3). Anal. calcd. for $C_{16}H_{19}NO_4PCl$: C, 54.02; H, 5.37; N, 3.93. Found: C, 53.92; H, 5.31; N, 3.88.

*[5-Methyl-2-hydroxyphenyl amino)-(4-dimethyl amino phenyl)-methyl]-phosphonic acid dimethyl ester (**4j**)*. Yield: 1.20 g, 66 %; m.p.: 210-212 °C. IR (KBr): ν (cm^{-1}) 3392 (-N-H); 1217 (-P=O); 752 (-P-$C_{aliphatic}$); 3402 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.44 (s, 1H, Ar-OH), 6.36-7.97 (m, 7H, aromatic); 4.91 (dd, $^2J_{PH}$ = 18.3 Hz, $^3J_{HH}$ = 9.6 Hz, P-C-H), 4.32 (t, 1H, $^3J_{PH}$ = 9.8 Hz, $^3J_{HH}$ = 9.1 Hz, PCNH), 3.43 (d, J_{POCH} = 10.6 Hz, P-OCH_3), 3.65 (d, J_{POCH} = 10.4 Hz, P-OCH_3), 2.36 (s, 1H, Ar-CH_3), 3.01 (s, 6H, R′4-N$(CH_3)_2$. ^{31}P NMR (DMSO-d_6): ppm 28.30. Anal. calcd. for $C_{18}H_{25}N_2O_4P$: C, 59.34; H, 6.91; N, 7.68. Found: C, 59.29; H, 6.89; N, 7.63.

*[5-Methyl-2-hydroxyphenyl amino)-(2-hydroxynaphthyl)-methyl]-phosphonic acid dimethyl ester (**4k**)*. Yield: 1.28 g, 68 %; m.p.: 282-284 °C. IR (KBr): ν (cm^{-1}) 3345 (-N-H); 1208 (-P=O); 745 (-P-$C_{aliphatic}$); 3426 (Ar-OH). ^{1}H NMR (DMSO-d_6): ppm 9.64 (s, 1H, Ar-OH), 6.95-7.91 (m, 9H, aromatic); 5.32 (dd, 1H, $^2J_{PH}$ = 24.2 Hz, $^3J_{HH}$ = 9.8 Hz, P-C-H), 5.12 (t, 1H, $^3J_{PH}$ = 9.2 Hz, $^3J_{HH}$ = 9.1 Hz, PCNH), 3.48 (d, J_{POCH} = 11.1 Hz, P-OCH_3), 4.59 (d, J_{POCH} = 12.2 Hz, P-OCH_3), 2.49 (s, 3H, Ar-CH_3). ^{31}P NMR (DMSO-d_6): ppm 36.01. Anal. calcd. for $C_{20}H_{22}NO_5P$: C, 62.02; H, 5.72; N, 3.61. Found: C, 61.89; H, 5.67; N, 3.55.

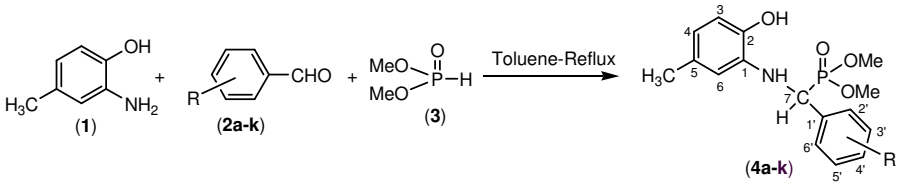
Biological activity

Bioassay. Agar well bioassay was employed for testing antibacterial and antifungal activity of **4a-4k**. Diluted inoculum 0.1 mL (10^5 CFU/mL) of bacteria was spread on nutrient agar and fungi on potato dextrose agar plates (PDA). Wells of 8 mm were punched into the agar medium and filled with the title compounds at the concentration of 25 and 50 µg in each well. The plates were incubated for 24 h at 37 °C for test bacteria and the fungi plates were incubated for 72 h at 28 °C. The antimicrobial activity was evaluated by measuring the zone of inhibition against test organisms. Chloramphenicol and Ketoconazole were used as commercial standards. Controls were maintained with dimethyl sulphoxide (DMSO) [17].

Determination of MIC. Minimum inhibitory concentration (MIC) was determined for the compounds that showed total growth inhibition using the protocol described below. The minimum concentration, at which there was no visually detectable bacterial growth, was taken as MIC. The compound concentration of 0.1 mg to 0.50 mg/mL in steps of 0.1 mg/mL was evaluated. Specifically 0.1 mL of standardized inoculum (1-2 x 10^7 CFU/mL) was added to each test tube. Two controls (DMSO with bacteria and antibiotics with bacteria) were maintained for each test sample. The tubes were incubated aerobically at 37 °C for 24 h. The method followed for antifungal bioassay is similar to that followed for antibacterial assay where in the medium is PDA and incubation temperature is 28 °C for 72 h [18].

RESULTS AND DISCUSSION

The synthetic route involves reaction of equimolar quantities of 2-amino-4-methyl phenol **1**, various aromatic aldehydes **2a-2k** and dimethylphosphite **3** in dry toluene at reflux temperature (Scheme 1).



Scheme 1

Compd.	R		Compd.	R
4a	4'-F		**4g**	4'-CH_3
4b	3'-NO_2		**4h**	4'-OMe
4c	4'-N (C_2H_5)$_2$		**4i**	4'-Cl
4d	4'-(CH_3)$_2$CH		**4j**	4' -N(CH_3)$_2$
4e	4'-(CH_3)$_3$C		**4k**	2'-$C_{10}H_6$- OH
4f	4'-OH			

Characteristic IR stretching frequencies are present in the region 3396-3432, 1202-1247, 745-771 and 3343-3446 cm^{-1} for Ar-OH, P=O, P-C $_{(aliphatic)}$ and N-H, respectively, in **4a-4k** [19, 20].

The aromatic hydrogens of the two benzene rings of α-aminophosphonates **4a-4k** showed a complex multiplet at 6.05-8.31 ppm. The P-C-H hydrogen signal appeared as doublet of doublet at 4.72-5.52 ppm due to its coupling with phosphorus and the hydrogen of N-H. The N-H proton showed triplet in the region 4.30-5.44 ppm for the compounds **4a-4k**, respectively.

The phenolic –OH group present in **1** gave signal at downfield region 9.12-9.81 ppm due to its acidic nature. Methoxy hydrogens linked to phosphorus showed two different doublets in the range one at 3.43-3.89 ppm (J = 9.56-11.56 Hz) and another at 3.52-4.59 ppm (J = 8.24-12.2 Hz) due to their magnetic non-equivalence. ^{13}C NMR spectra for a few representative compounds (**4a, 4b, 4c, 4h, 4i,**) showed carbon chemical shifts in the expected region. ^{31}P NMR chemical shifts appeared in the region 18.59-36.01 ppm for all the compounds **4a-4k** [20]. In Kabachnik–Fields reaction, the amine and hydrophosphoryl compound form a complex in which one of the partners reacts with the carbonyl compound at reflux in toluene also favors the formation of α-aminophosphonate. Another important feature of this reaction is the robustness of functional groups, such as fluoro, chloro and hydroxyl groups under reaction conditions. It is worth mentioning that synthesized α-aminophosphonates are novel and inaccessible to preparation by other methods.

FAB mass spectra of **4a, 4b** and **4h** showed $M^{+\bullet}$ ions and respective characteristic daughter ions. Agar well method was employed for screening antibacterial and antifungal activities. **4a** showed broad spectrum activity with highest zone of inhibition (Table 1) and was found to inhibit the growth of isolated bacteria and fungi with lowest MIC value (Table 2). The previous results are also correlated with present investigation [21, 22].

Table 1. Diameter of inhibition zone (mm) of α-amino phosphonates **4a-4k**.

Entry	*Staphyloco-ccus aureus*		*Bacillus subtilis*		*Escherichia coli*		*Klebsiella pneumoniae*		*Salmonella typhimurium*		*Candida albicans*		*Aspergillus niger*	
	25	50	25	50	25	50	25	50	25	50	25	50	25	50
4a	12	15	12	15	12	15	12	15	12	15	10	12	10	12
4b	10	12	10	12	-	12	-	12	-	12	-	10	-	10
4c	-	12	-	12	-	12	-	12	-	12	-	10	-	10
4d	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4e	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4f	10	12	10	12	-	10	-	10	-	10	-	10	-	10
4g	10	12	10	12	-	12	-	12	-	12	-	10	-	10
4h	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4i	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4j	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4k	-	10	-	10	-	10	-	10	-	10	-	-	-	-
Chloraemphenicol (5)	-	25	- 26		-	22	-	30	-	22	-	-	-	-
Ketoconazole (50)	-	-	-	-	-	-	-	-	-	-	-	16	-	18

Each well contains 25 and 50 μg of compounds. Chloraemphenicol 5 μg/well, Ketoconazole 50 μg/well.

Table 2. Minimal inhibitory concentration (MIC, mg/mL) of **4a-4k**.

Entry	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhimurium*	*Candida albicans*	*Aspergillus niger*
4a	0.16	0.17	0.22	0.21	0.20	0.29	0.27
4b	0.28	0.31	0.30	0.31	0.32	0.33	0.34
4c	0.32	0.31	0.36	0.33	0.31	0.34	0.37
4d	0.42	0.44	0.40	0.47	0.41	-	-
4e	0.46	0.48	0.44	0.44	0.39	-	-
4f	0.30	0.32	0.32	0.29	0.30	0.40	0.38
4g	0.29	0.30	0.30	0.33	0.321	0.33	0.34
4h	0.41	0.40	0.50	0.50	0.47	-	-
4i	0.42	0.44	0.44	0.43	0.41	-	-
4j	0.45	0.46	0.40	0.40	0.44	-	-
4k	0.40	0.42	0.42	0.41	0.39	-	-

CONCLUSIONS

In our endeavour, a simple procedure for the synthesis of new α-aminophosphonates **4a-4k** in high yields by Kabachnik-Fields reaction without using any catalyst is described. All the synthesized compounds showed moderate antibacterial activity against both gram negative and gram positive bacteria.

AKNOWLEDGEMENTS

The authors thanks to Prof. C.D. Reddy, Department of Chemistry, S.V. University, Tirupati (India) for helpful discussions and for BRNS (DAE), Mumbai for providing financial assistance.

REFERENCES

1. Barlett, P.A.; Hanson, J.E.; Giannousis, P.P. *J. Org. Chem*. **1990**, 55, 6268.
2. Wang, H.L.; Zhou, J.; Qiu, Y.G.; Feng, K.S.; Chen, R.Y. *Phosphorus, Sulfur, Silicon* **1995**, 104, 135.
3. (a) Zhou, J.; Chen, R.Y.; Yang, X.F. *Heteroatom Chem.* **1998**, 9, 369. (b) Zhou, J.; Chen, R.Y. *J. Chem. Res. (S)* **1998**, 5, 254. (c) Zhou, J.; Chen, R.Y. *Synth. Commun.* **1998**, 28, 2653.
4. (a) Kafarski, P.; Lejczak, B. *Curr. Med. Chem. Anticancer Agents* **2001**, 1, 301. (b) Kiran, Y.B.; Reddy, C.D.; Gunasekar, D.; Suresh Reddy, C.; Barbosa, L.C.A.; Annette L. *Eur. J. Medicinal Chem.* **2008**, 43, 885.
5. De Lombaert, S.; Blanchard, L.; Tan, J.; Sankane, Y.; Berry, C.; Ghai, R.D. *Bioorg. Med. Chem. Lett.* **1995**, 5, 145.
6. Kafarski, P.; Lejczak, B. *Phosphorus, Sulfur, Silicon, Relat. Elem.* **1991**, 63, 193.
7. Atherton, F.R.; Hassall, C.H.; Lambert, R.W. *J. Med. Chem.* **1986**, 29, 29.
8. (a) Kukhar, V.P.; Solodenko, V.A. *Rus. Chem. Rev (Engl. Trans.)* **1987**, 56, 859. (b) Yokomatsu, T.; Yoshida, Y.; Shibuya, S. *J. Org. Chem.* **1994**, 59, 7930.
9. Chandrasekhar, S.; Narsihmulu, Ch.; Shameen Sultana, S.; Saritha, B.; Jayaprakash, S. *Synlett.* **2003**, 4, 505.
10. Takahashi, H.; Yoshioka, M.; Imai, N.; Onimura, K.; Kobayashi, S. *Synthesis* **1994**, 8, 763.
11. Heydari, A.; Karimian, A.; Ipaktschi, J. *Tetrahedron Lett.* **1998**, 39, 6729.
12. Azizi, N.; Saidi, M.R. *Eur. J. Org. Chem.* **2003**, 23, 4630.
13. Chandrasekhar, S.; Prakash, S.J.; Jagadeshwar, V.; Narsihmulu, Ch. *Tetrahedron Lett.* **2001**, 42, 5561.
14. Ranu, B.C.; Hajra, A.; Jana, U. *Org. Lett.* **1999**, 1, 1141.
15. Lee, S.; Park, J.H.; Kang, J.; Lee, J.K. *Chem. Commun.* **2001**, 42, 8441.
16. Akiyama, T.; Sanada, M.; Fuchibe, K. *Synlett.* **2003**, 10, 1463.
17. Mangte, D.V.; Deshmukh, S.P.; Bhokare, D.D.; Deshpande Arti, R. *Ind. J. Pharma.* **2007**, 69, 295.
18. Erturk, O. *Biologia* **2006**, 61, 275.
19. Devendranath Reddy, C.; Reddy, M.S.; Raju, C.N. *Ind. J. Chem.* **2000**, 39B, 426.
20. Quin, L.D.; Verkade, J.G. *Phosphorus-31 NMR Spectral Properties Compound Characterization and Structural Analysis*, VCH Publisher: New York; **1994**.
21. Sankar, A.U.R.; Kumar, B.S.; Raju, C.N.; Reddy, C.S. *S. Afr. J. Chem.* **2007**, 60, 125.
22. Srinivasulu, K.; Raju, C.N.; Babu, H.Y.; Reddy, L.A.V. *S. Afr. J. Chem.* **2007**, 60, 47.