# Microwave assisted greener approach to synthesize α-aminophosphonates under catalyst free and solvent-free condition and their bioactivity evaluation

**D. Ravikumar[1], Ch. Subramanyam[2], S. Mohan[3] and K. Prasada Rao[4*]**

**Research Scholar[1], Assistant Professor[2,3], Sr. Assistant Professor[4], Department of Chemistry, Bapatla Engineering College, Bapatla, India.**

***ravikumardone@gmail.com[1], subbusree04@gmail.com[2], seelam.mohan123@gmail.com[3], prasad17467@gmail.com[4]***

**Abstract. Microwave promoted easy, efficient and environment friendly procedure has been devised for the synthesis of α-aminophosphonates in high yields through a one-pot three-component condensation (Kabachnik-Fields reaction) of the amine, substituted aldehydes and triethylphophite under catalyst free, solvent free conditions. The newly synthesized compounds were characterized by NMR ($^{31}$P, $^{1}$H and $^{13}$C), Mass, IR and C, H, N analyses. All the newly synthesized compounds were screened for their antibacterial and antifungal activities. Majority of the compounds showed good activity against both bacterial and fungal strains.**

***Keywords* — microwave irradiation, α-aminophosphonates, Kabachnik-Fields reaction, antibacterial and antifungal activities**

## I. INTRODUCTION

Organophosphorus compounds have been found a wide range of applications in the areas of industrial, agricultural and medicinal chemistry owing to their biological and physical properties as well as their utility as synthetic intermediates.[1-5] α-Aminophosphonates have been the focus of attention in recent years because of their structural analogy to the corresponding α-amino acids. These molecules have found a wide range of applications in the areas of industrial, biological and medicinal chemistry as inhibitors of synthase, HIV protease,[6a] anti-thrombotic agents,[6b] insecticides,[6c] plant growth regulators[6d,e] antitumor,[6f] antiviral,[6g,h,i] antibacterial,[6j] antifungal,[6k] anti-HIV,[6l] anti-inflammatory[6m] antioxidant[6n] and herbicidal activities.[6o]

Hence a large number of synthetic routes have been developed for the synthesis and evolution of bioactivity of α-aminophosphonates and their derivatives. Kabachnik-Field's reaction is one of the most versatile pathways for the formation of carbon-phosphorus bonds. It involves one pot three component reaction of aldehyde, amine and dialkyl phosphite[7] or trialkylphosphite.[8] Recently several reports are published describing the synthesis of α-aminophosphonates *via* Kabachnik-Fields reaction using Lewis acids,[9] Bronsted acids,[10] solid acids,[11] bases,[12] nano catalysts[13] and other catalysts.[14]Although these approaches are satisfactory for a one-pot synthesis of α-aminophosphonates, they suffer from at least one of the following drawbacks such as long reaction times, low yields of the products requiring stoichiometric amounts of catalysts, excess amounts of phosphorus compounds or use of additives. In recent years, microwave assisted organic syntheses have gained enormous attention of the chemists due to their advantages such as shorter reaction times, cleaner products, operational simplicity, higher yields and being a potential alternative to accomplish the effective synthesis of heterocyclic bioactive compounds.[15] Solvent-free reaction condition has been demonstrated to be an efficient technique for various organic reactions. It often leads to a remarkable decrease in reaction time, increased yields, easier workup, enhancement of regio and stereo selectivity of reaction matches with the green chemistry protocol.[16]

By considering the above facts and in continuation of our studies towards developing new methods for the synthesis of α-aminophosphonates, we decided to explore the possibility of implementing a one-pot three-component reaction for the preparation of α-aminophosphonates.

## II. EXPERIMENTAL DETAILS

The chemicals are procured from Sigma-Aldrich, Merck and Lancaster were used as such without further purification. The reagent grade solvents were used for spectroscopic and other physical studies and were further purified employing the reported methods. The melting points were determined in open capillary tubes on a Guna Digital Melting Point apparatus and are uncorrected. The

$^{31}$P, $^{1}$H, $^{13}$C and NMR Spectra were recorded on Bruker AMX spectrometer operating at 161.9 MHz for $^{31}$P, 400 MHz for $^{1}$H and 100 MHz for $^{13}$C NMR. All compounds were dissolved in DMSO-$d_6$ and chemical shifts were referenced to TMS ($^{1}$H and $^{13}$C NMR) and 85% $H_3PO_4$ ($^{31}$P NMR) and Mass spectra were recorded on API 2000 Perkin-Elmer PE-SCIEX Mass spectrometer. Micro-analytical data were obtained from University of Hyderabad, Hyderabad, India.

***Procedure for the synthesis of α-aminophosphonates 4a-j***

A mixture of benzaldehyde (0.01 mol), 2, 4-dichloroaniline (0.01 mol), triethyl phosphite (0.02 mol) were taken in flat-bottomed flask and irradiated with microwave radiations in a microwave oven at 490 Watts. The reaction mixture was heated consecutively two times for 2-3 min period each time followed by a 1 min cooling interval between irradiations. To avoid the continuous overheating of the reactants, this method was proposed. In order to maintain the homogeneity of the irradiating field throughout the reaction, the reaction mixture was kept under stirring. The progress of the reaction was monitored by TLC on silica gel using ethyl acetate-hexane (6:4 v/v). After complection of the reaction, the solvent was removed under reduced pressure to get the crude product. The resulting crude product was purified by column chromatography on silica gel (100-200 mesh) using ethyl acetate-hexane (1:1) as eluent to get pure diethyl (2,4-dichlorophenylamino)(phenyl)methylphosphonate (**4a**). The remaining compounds **4b-j** were prepared by adapting to the above described procedure. The synthetic protocol for the title compounds is presented in **Scheme 1**.

***Physical, analytical and spectral data for the compounds (4a-j)***

Diethyl (2,4-dichlorophenylamino)(phenyl)methylphosphonate (4a): Semi solid.; $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 17.0 ppm; $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 8.17-6.42 (m, 8H, Ar-H), 5.28 (s, 1H, C-NH), 4.48 (d, 1H, P-CH), 4.02 (m, 4H, O-$\underline{CH_2}CH_3$), 1.21 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 143.1, 138.3, 131.9, 129.7, 128.8, 126.8, 125.5, 123.6, 123.1, 114.3, 63.7, 56.7, 16.8; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3328 (NH), 742 (P-$C_{aliphatic}$), 1220 (P=O); LCMS (m/z, %): 388 ($M+H^+$, 100), 390 (M+2, 64); Anal. Calcd for $C_{17}H_{20}Cl_2NO_3P$: C, 52.59; H, 5.19; N, 3.61%; found: C, 52.65; H, 5.10; N, 3.66%.

diethyl (2,4-dichlorophenylamino)(4-fluorophenyl)methylphosphonate (4b): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 17.8 ppm; $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 7.95-6.42 (m, 7H, Ar-H), 5.29 (s, 1H, C-NH), 4.51(d, 1H, P-CH), 4.09 (m, 4H, O-$\underline{CH_2}CH_3$), 1.26 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 162.1, 143.1, 131.9, 129.7, 128.8, 126.8, 125.5, 117.2, 114.3, 63.9, 56.8, 16.8; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3343 (NH), 762 (P-$C_{aliphatic}$), 1228 (P=O); LCMS (m/z, %): 406 ($M+H^+$, 100); Anal. Calcd for $C_{17}H_{19}Cl_2FNO_3P$: C, 50.26; H, 4.71; N, 3.45%; found: C, 50.32; H, 4.79; N, 3.51%.

diethyl (2,4-dichlorophenylamino)(4-nitrophenyl)methylphosphonate (4c): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 18.6 ppm; $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 8.17-6.42 (m, 8H, Ar-H), 5.28 (s, 1H, C-NH), 4.59 (d, 1H, P-CH), 4.09 (m, 4H, O-$\underline{CH_2}CH_3$), 1.27 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 147.1, 143.3, 138.3, 129.7, 128.8, 126.8, 125.5, 123.6, 123.1, 114.3, 63.7, 56.7, 16.8; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3354 (NH), 753 (P-$C_{aliphatic}$), 1227 (P=O); LCMS (m/z, %): 433 ($M+H^+$, 100), 435 (M+2, 64); Anal. Calcd for $C_{17}H_{19}Cl_2N_2O_5P$: C, 47.13; H, 4.42; N, 6.47%; found: C, 47.25; H, 4.35; N, 6.54%.

diethyl (2,4-dichlorophenylamino)(4-methoxyphenyl)-methylphosphonate (4d): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 16.7 ppm; $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 8.04-6.42 (m, 7H, Ar-H), 5.40 (s, 1H, C-NH), 4.59 (d, 1H, P-CH), 4.01 (m, 4H, O-$\underline{CH_2}CH_3$), 3.42 (s, 1H, $OCH_3$), 1.30 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 155.4, 143.3, 129.7, 128.8, 127.6, 126.8, 125.5, 123.6, 123.1, 114.3, 63.5, 57.2, 56.4, 16.6; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3327 (NH), 740 (P-$C_{aliphatic}$), 1221 (P=O); LCMS (m/z, %): 418 ($M+H^+$, 100), 420 (M+2, 64); Anal. Calcd for $C_{18}H_{22}Cl_2NO_4P$: C, 51.69; H, 5.30; N, 3.35%; found: C, 51.75; H, 5.35; N, 3.28%.

diethyl (4-chlorophenyl)(2,4-dichlorophenylamino)methylphosphonate (4e): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 17.6 ppm; $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 7.94-6.43 (m, 7H, Ar-H), 5.42 (s, 1H, C-NH), 4.55 (d, 1H, P-CH), 4.03 (m, 4H, O-$\underline{CH_2}CH_3$), 1.26 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 145.6, 136.3, 134.7, 129.8, 128.5, 127.6, 126.8, 125.5, 123.6, 123.1, 114.3, 63.7, 56.5, 16.7; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3367 (NH), 755 (P-$C_{aliphatic}$), 1225 (P=O); LCMS (m/z, %): 422 ($M+H^+$, 100), 424 (M+2, 96), 426 (M+4, 31); Anal. Calcd for $C_{17}H_{19}Cl_3NO_3P$: C, 48.31; H, 4.53; N, 3.31%; found: C, 48.39; H, 4.58; N, 3.26%.

(E)-diethyl 1-(2,4-dichlorophenylamino)-3-phenylallyl-phosphonate (4f): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 16.4 ppm; $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 7.94-6.43 (m, 8H, Ar-H), 6.42 (d, 1H, Ar-C**H**=CH-), 6.05 (m, 1H, Ar-CH=C**H**-), 5.35 (s, 1H, C-NH), 4.42 (d, 1H, P-CH), 4.01 (m, 4H, O-$\underline{CH_2}CH_3$), 1.23 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 144.1, 136.3, 132.4, 129.8, 128.5, 127.6, 127.2, 126.8, 125.5, 123.6, 123.1, 114.3, 63.7, 56.4, 16.7; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3352 (NH), 749 (P-$C_{aliphatic}$), 1224 (P=O); LCMS (m/z, %): 414 ($M+H^+$, 100), 416 (M+2, 64); Anal. Calcd for $C_{19}H_{22}Cl_2NO_3P$: C, 55.09; H, 5.35; N, 3.38%; found: C, 55.16; H, 5.48; N, 3.31%.

diethyl (2,4-dichlorophenylamino)(3-nitrophenyl)methylphosphonate (4g): Semi solid. $^{31}$P NMR spectrum (DMSO-

$d_6$): $\delta$ 17.7 ppm; $^1$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 8.14-6.65 (m, 7H, Ar-H), 5.42 (s, 1H, C-NH), 4.74 (d, 1H, P-CH), 4.02 (m, 4H, O-$\underline{CH_2}CH_3$), 1.19 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 149.2, 144.5, 136.3, 132.4, 129.8, 128.5, 127.6, 126.8, 125.5, 123.6, 123.1, 114.3, 63.7, 56.4, 16.7; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3379 (NH), 761 (P-$C_{aliphatic}$), 1229 (P=O); LCMS (m/z, %): 433 (M+H$^+$, 100), 435 (M+2, 64); Anal. Calcd for $C_{17}H_{19}Cl_2N_2O_5P$: C, 47.13; H, 4.42; N, 6.47%; found: C, 47.19; H, 4.36; N, 6.49%.

diethyl (3-chlorophenyl)(2,4-dichlorophenylamino)methyl-phosphonate (4h): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 18.4 ppm; $^1$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 8.15-6.64 (m, 7H, Ar-H), 5.41 (s, 1H, C-NH), 4.72 (d, 1H, P-CH), 4.02 (m, 4H, O-$\underline{CH_2}CH_3$), 1.18 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 149.2, 136.3, 133.5, 132.4, 129.8, 128.5, 127.6, 126.8, 125.5, 123.6, 123.1, 114.3, 63.7, 56.4, 16.7; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3341 (NH), 758 (P-$C_{aliphatic}$), 1227 (P=O); LCMS (m/z, %): 422 (M+H$^+$, 100), 424 (M+2, 96), 424 (M+4, 31); Anal. Calcd for $C_{17}H_{19}Cl_3NO_3P$: C, 48.31; H, 4.53; N, 3.31%; found: C, 47.19; H, 4.36; N, 6.49%.

diethyl (2,4-dichlorophenylamino)(5-nitrothiophen-2-yl)-methylphosphonate (4i): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 19.2 ppm; $^1$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 8.23-6.63 (m, 5H, Ar-H), 5.47 (s, 1H, C-NH), 4.76 (d, 1H, P-CH), 4.03 (m, 4H, O-$\underline{CH_2}CH_3$), 1.18 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 152.4, 149.2, 144.3, 129.8, 128.5, 127.6, 125.5, 123.6, 123.1, 114.4, 63.8, 56.8, 16.9; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3347 (NH), 746 (P-$C_{aliphatic}$), 1232 (P=O); LCMS (m/z, %): 439 (M+H$^+$, 100); Anal. Calcd for $C_{15}H_{17}Cl_2N_2O_5PS$: C, 41.02; H, 3.90; N, 6.38%; found: C, 41.10; H, 3.84; N, 6.44%.

diethyl (2,4-dichlorophenylamino)(6-methoxypyridin-2-yl)methylphosphonate (4j): Semi solid. $^{31}$P NMR spectrum (DMSO-$d_6$): $\delta$ 19.5 ppm; $^1$H NMR spectrum (400 MHz, DMSO-$d_6$)**:** $\delta$ 8.15-6.65 (m, 6H, Ar-H), 5.45 (s, 1H, C-NH), 4.73 (d, 1H, P-CH), 4.01 (m, 4H, O-$\underline{CH_2}CH_3$), 3.45 (s, 1H, $OCH_3$), 1.18 (t, $J$ = 7.2 Hz, 6H, O-$CH_2\underline{CH_3}$); $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$): $\delta$ 162.4, 154.6, 144.3, 137.3, 129.8, 128.5, 127.6, 125.5, 123.6, 123.1, 114.4, 63.8, 56.8, 53.3, 16.9; IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3338 (NH), 753 (P-$C_{aliphatic}$), 1223 (P=O); LCMS (m/z, %): 419 (M+H$^+$, 100), 421 (M+2, 64); Anal. Calcd for $C_{17}H_{21}Cl_2N_2O_4P$: C, 48.70; H, 5.05; N, 6.68%; found: C, 48.76; H, 5.01; N, 6.74%.

## III. BIOLOGICAL ACTIVITY

### *Antibacterial Activity*

The bacterial strains *Bacillus subtilis* (ATCC-23857), *Staphylococcus aureus* (ATCC-19433) (Gram positive) and *Escherichia coli* (ATCC-10148), *Klebsiella pneumoniae* (ATCC-31488) (Gram negative) were selected to screen the antibacterial activity of the title compounds by using agar well diffusion method.[17] Penicillin was used as a standard drug for antibacterial studies. A standard inoculums of 1.2 × 10$^{-7}$ c.f.u/mL (0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. Discs measuring 6mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140 °C for an hour. The dry sterilized discs soaked in a known concentration (100 $\mu$g/mL) of the test compounds were placed in nutrient agar medium. Blank test showed that DMSO used in the preparation of the test solutions does not affect the bacteria. The plates after inoculation were inverted and incubated for 24 h at 37 °C. The zone of inhibition around the disc which was calculated edge to edge zone of the confluent growth usually corresponds to the sharpest edge of the zone and was measured in millimeters. All tests were repeated three times and average data was taken as final result. Minimum inhibitory concentrations (MICs) were also determined by microbroth dilution technique. Specifically 0.1mL of standardized inoculum (1.2 × 10$^{-7}$ c.f.u/mL) was added to test tubes and incubated for 24 h at 37 °C and two controls were maintained for each test sample. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC) (**Table 4**).

### *Antifungal bioassay*

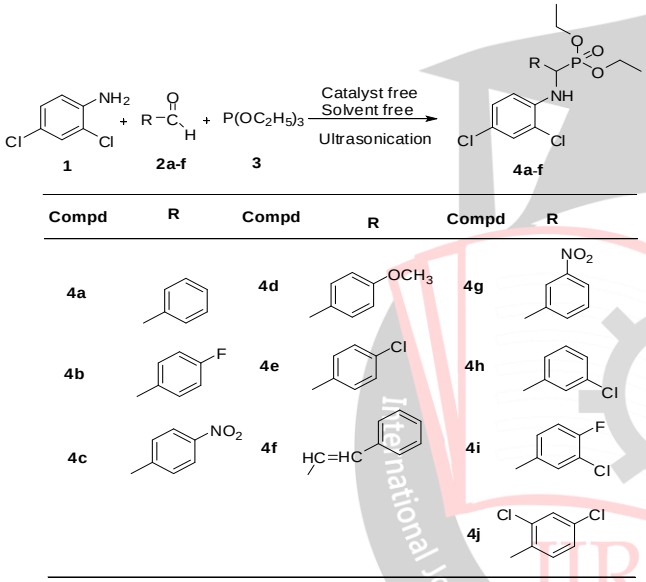
The fungal strains *Helminthosporium oryzae* (ATCC 11000) and *Aspergillus niger (*ATCC 16404) were selected to screen the antifungal activity of the newly synthesized compounds by agar disc-diffusion method.[18] The fungal strains were maintained on potato dextrose agar (PDA) medium (Hi-Media). A loop full of culture from the slant was inoculated into the potato dextrose broth and incubated at 37 ºC for 48-72 h. 0.1 mL of this culture was spread on the potato dextrose agar plate using a sterile glass spreader for even spreading of the inoculum. Sterile discs of Whatmann No.1 filter paper of about 6 mm diameter were impregnated on the surface of the media. All the compounds were dissolved in dimethyl sulfoxide (DMSO). Sterile discs of Whatman No. 1 filter paper of about 6mm diameter were dried and soaked in 100 $\mu$g/mL concentration of the test samples. The soaked discs were impregnated on the surface of the media and incubated for 48–72 h at 37 ºC. Blank test showed that DMSO used in the preparations of the test solutions does not affect the test fungi. The zone of inhibition around the disc was the calculated edge to edge zone of the confluent growth which usually corresponds to the sharpest edge of the zone and was measured in millimeters. All tests were repeated three times and average data was taken as final result. *Griseofulvin* was used as a standard drug for antifungal study. Minimum Inhibitory Concentration (MIC) of the tested samples was determined by micro-broth-dilution method [27–29]. Specifically 0.1mL of standardized

inoculum (1-2 × 107 c.f.u/mL) was added to each test tube. The tubes were incubated aerobically at 37 ºC for 48–72 h. Control was maintained for each test sample. The lowest concentrations (highest dilution) of test compound that produced no visible signs of fungal growth (no turbidity) when compared with the control tubes were regarded as MICs (**Table 5**).

## IV. RESULTS AND DISCUSSION

### *Chemistry*

In this letter, we report an efficient and environmentally benign protocol for the synthesis of *α*-aminophosphonates by the reaction of 2, 4-dichloroaniline (0.01 mol), various aldehydes (0.01 mol) and triethyl phosphite (0.02 mol) under solvent-free conditions at room temperature using microwave radiation at 490W *via* Kabachnik-Fields reaction (**Scheme 1**).




**Scheme 1: Synthesis of some novel *α*-aminophosphonates (4a-j)**

To optimize the reaction conditions, the reaction was demonstrated with models such as 2, 4- dichloroaniline (0.01 mol), various aldehydes (0.01 mol) and triethyl phosphite (0.02 mol). It was observed that, the reaction proceeded efficiently to afford the corresponding *α*-aminophosphonate within shorter time (10 min.) at room temperature (**Table 1**, **entry 3**). The products were purified through recrystallization without applying any chromatographic technique. This avoids use of large quantities of volatile organic solvents usually required for work-up and purification in many existing procedures.

Further, the reaction was carried out with and without solvents at room temperature exclusive of exposing to microwave radiations, only traces of the product were observed even after 24 h (**Table 1**, **entry 1-2**). In the absence of microwave radiations increased temperature also could not enhance the yield of the product (**Table 1**, **entry 5-6**). Obviously, the use of microwave radiations under solvent free condition might be the key factor to the high efficiency of the one-pot reactions.

| Entry | Reaction Condition | Solvent | Time | Yield[a] (%) |
|---|---|---|---|---|
| 1 | Room temperature without Microwave irradiation | THF | 24h | Traces |
| 2 | Room temperature without Microwave irradiation | No solvent | 24h | Traces |
| 3 | Room temperature with Microwave irradiation | No solvent | 10 min | 90 |
| 4 | Room temperature with Microwave irradiation | THF | 10 min | 72 |
| 5 | Reflux without Microwave irradiation | No solvent | 3h | 20 |
| 6 | Reflux without Microwave irradiation | THF | 3h | 35 |

**Table 1.** Reaction of 2, 4- dichloroaniline, benzaldehyde and triethyl phosphite under different reaction conditions

[a]Isolated Yield

Further, we studied the effect of solvents on model reaction. Here, we used solvents like THF, methanol, dichloromethane, toluene hexane, acetonitrile and ethanol. The scrutiny revealed that in all the solvents the yields of the products were found to be 58-75% (**Table 2**, **entry 2-8**) after 3h. When we compared these results with the results obtained under solvent free condition, the later condition proved to be better reaction condition to obtain high yield (90%) of the product (**Table 2**, **entry 1**).

**Table 2**: Effect of Solvents on the condensation of 2, 4-dichloroaniline, various aldehydes and triethylphosphite under microwave irradiation

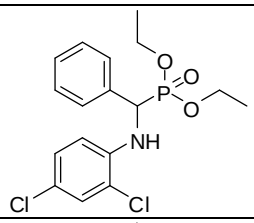
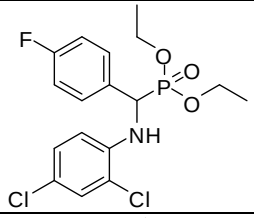
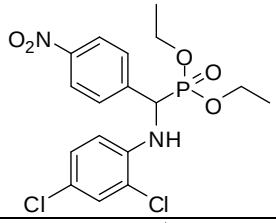
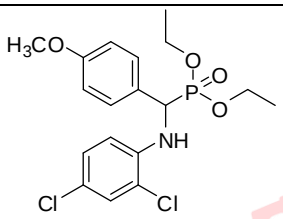
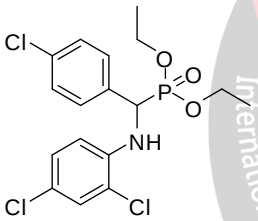
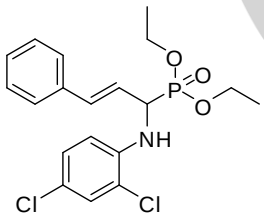
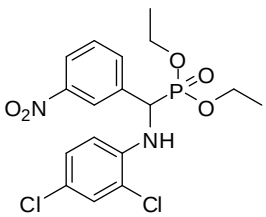
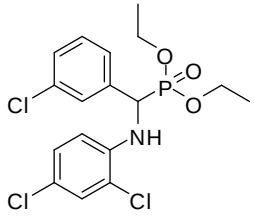
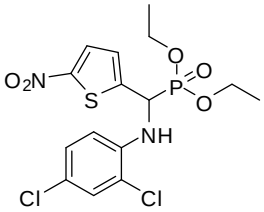
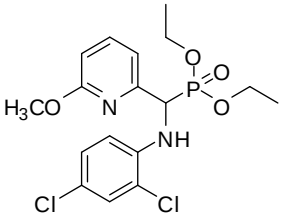
| Entry | Solvent | Time (min) | Yield[a] (%) |
|---|---|---|---|
| 1 | Solvent-free | 10 | 90 |
| 2 | Tetrahydrofuran (THF) | 10 | 72 |
| 3 | Methanol | 10 | 74 |
| 4 | Dichloromethane | 10 | 69 |
| 5 | Toluene | 10 | 70 |
| 6 | Hexane | 10 | 58 |
| 7 | Acetonitrile | 10 | 73 |
| 8 | Ethanol | 10 | 75 |

[a]Isolated Yield

After optimizing the conditions, the generality of this method for the synthesis of *α*-aminophosphonates (**4a-j**) (**Scheme 1**) was examined by the reaction of several aromatic and heterocyclic aldehydes (**3b-j**), amine (**2**) and triethyl phosphite (**4**) using irradiation radiation under solvent free conditions and the results were summarized in

**Table 3.** Microwave assisted synthesis of *α*-aminophosphonates (**4a-j**) under solvent free and catalyst free conditions

| Compd | Structure | Time | Yield[a] |
|---|---|---|---|

| | | (min) | |
|---|---|---|---|
| **4a** |  | 10 | 96 |
| **4b** |  | 12 | 95 |
| **4c** |  | 12 | 97 |
| **4d** |  | 15 | 89 |
| **4e** |  | 10 | 92 |
| **4f** |  | 10 | 90 |
| **4g** |  | 18 | 92 |
| **4h** |  | 14 | 92 |
| **4i** |  | 11 | 93 |
| **4j** |  | 15 | 91 |

[a]Isolated Yield

The chemical structure of the title compounds **4a-j** were characterized by spectral data ($^{31}$P, $^{1}$H and $^{13}$C NMR, IR and LC-MS), elemental analysis and the results were presented in experimental section. $^{31}$P NMR signals were observed in the region 19.5-16.4 ppm for all the compounds **4a–j.** The $^{1}$H NMR spectra gave signals as multiplet due to Ar-H in the range of $\delta$ 8.23-6.42 ppm. The proton signals in the range of 5.47-5.28 and 4.76-4.48 ppm were due to C-NH and P-CH respectively. The methylene protons of P-O-C**H**$_2$CH$_3$ gave a multiplet and methyl protons of P-O-CH$_2$C**H**$_3$ resonated as a triplet in the region $\delta$ 4.09-4.01 and $\delta$ 1.30-1.18 respectively for the compounds **4a-j**. $^{13}$C NMR chemical shift for P-CH, methylene and methyl carbons were observed in the region 56.8-56.4, 63.8-63.5 and 16.9-16.6 ppm respectively for the compounds **4a-j**. IR absorptions in the regions 3399-3380, 1502-1485 and 1248-1225 cm$^{-1}$ were assigned to indazole-NH, NH and P=O stretching vibrations respectively for the compounds **4a-j**. In their mass spectra, $M^{+\cdot}$ ions were observed in the expected m/z values.

**Biological activity**

**Antibacterial Activity**

Two Gram positive bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*) and Two Gram negative bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*) were selected to screen the antibacterial activity of the title compounds by using agar well diffusion method.[17] Penicillin was used as a standard drug for antibacterial studies. Minimum inhibitory concentrations (MICs) were also determined by microbroth dilution technique. All title compounds showed promising activity against the bacterial strains at 100 µg/mL concentration.

Especially the compound **4b** (MIC= 10-25 $\mu$g/mL) bearing 4-fluorophenyl moiety and **4i** (MIC= 10-15 $\mu$g/mL) bearing 5-nitrothiophen-2-yl moiety showed slightly higher ZOI against all bacterial strains when compared with the standard drug. The compounds **4c**, bearing with 4-nitrophenyl moiety; **4e,** bearing 4-chlorophenyl moiety; **4h**

bearing 3-chlorophenyl moiety and **4j** bearing with 6-methoxypyridin-2-yl moiety exhibited good activity against all bacterial strains. The remaining compounds showed moderate activity when compared with standard bactericide.

**Table 4**. Antibacterial activity of $\alpha$-aminophosphonates (**4a-j**)

| Compd. | Gram positive bacteria | | | | Gram negative bacteria | | | |
|---|---|---|---|---|---|---|---|---|
| | ***B. subtilis*** | | ***S. aureus*** | | ***E. coli*** | | ***K. pneumoniae*** | |
| | ZOI[b] | MIC | ZOI[b] | MIC | ZOI[b] | MIC | ZOI[b] | MIC |
| 4a | 11.3 | 2 | 6.3 | 35 | 8.3 | 75 | 8.1 | 80 |
| 4b | 16.9 | 10 | 11.2 | 15 | 12.9 | 15 | 15.6 | 25 |
| 4c | 14.7 | 15 | 9.5 | 25 | 12.2 | 20 | 14.8 | 20 |
| 4d | 11.5 | 20 | 8.2 | 15 | 10.3 | 35 | 7.3 | 50 |
| 4e | 15.2 | 12 | 10.5 | 15 | 13.4 | 18 | 13.8 | 15 |
| 4f | 9.3 | 30 | 6.7 | 50 | 10.6 | 45 | 11.4 | 55 |
| 4g | 12.8 | 25 | 7.9 | 35 | 9.9 | 30 | 14.6 | 20 |
| 4h | 14.8 | 15 | 8.7 | 15 | 9.4 | 25 | 12.8 | 25 |
| 4i | 16.4 | 10 | 11.5 | 12 | 12.9 | 15 | 16.2 | 10 |
| 4j | 14.3 | 15 | 9.4 | 25 | 10.1 | 30 | 13.8 | 25 |
| [a]Std. | 16.1 | 4 | 10.4 | 6 | 12.3 | 5 | 15.2 | 4 |
| Control (DMSO) | - | - | - | - | - | - | - | - |

[a]Std.: Penicillin

[b]Concentration of compounds at 100 $\mu$g/mL.

ZOI: zone of inhibition in mm.

***Antifungal Activity***

All the synthesized compounds were screened for their antifungal activity against two fungal strains *Helminthosporium oryzae and Aspergillus niger* by agar disc-diffusion method.[18] Especially the compound **4b** (MIC= 10-15 $\mu$g/mL) bearing 4-fluorophenyl moiety showed slightly higher ZOI against all fungal strains when compared with the standard drug. The compounds **4e**, substituted with 4-chlorophenyl group; **4h**, substituted with 3-chlorophenyl group and **4i** bearing with 5-nitrothiophen-2-yl moiety exhibited good antifungal activity against all fungal strains.

**Table 5**. Antifungal activity of $\alpha$-aminophosphonates (**4a-j**)

| Compd | Fungal strains | | | |
|---|---|---|---|---|
| | *H. oryzae* | | *A. niger* | |
| | ZOI[b] | MIC | ZOI[b] | MIC |
| 4a | 8.3 | 40 | 9.8 | 24 |
| 4b | 13.2 | 15 | 13.9 | 10 |
| 4c | 7.7 | 50 | 9.2 | 30 |
| 4d | 5.2 | 80 | 6.6 | 75 |
| 4e | 12.1 | 18 | 12.5 | 15 |
| 4f | 7.8 | 45 | 8.3 | 40 |
| 4g | 9.6 | 30 | 7.8 | 35 |
| 4h | 11.9 | 20 | 12.2 | 18 |
| 4i | 11.5 | 25 | 12.0 | 20 |
| 4j | 10.1 | 27 | 10.7 | 25 |
| [a]Std. | 12.2 | 5 | 12.9 | 5 |
| Control (DMSO) | - | - | - | - |

[a] *Std*: *Griseofulvin*

[b]Concentration of compounds at 100 $\mu$g/mL; ZOI: zone of inhibition in mm.

## V. CONCLUSION

In conclusion, a series of new $\alpha$-aminophosphonates were synthesized in high yields under solvent free and catalyst free condition using microwave irradiation *via* Kabachnik-Field's reaction. All the newly synthesized compounds were screened *in vitro* for their antibacterial and antifungal activity against four bacterial strains and two fungal strains. The compounds **4b**, **4e**, **4h**, **4i** and **4j** showed potent activity against both bacterial and fungal strains. The high activity of the compounds might be due to the presence of electron with substituents like F, Cl and $NO_2$ and pyridine moiety.

## ACKNOWLEDGMENTS

The authors acknowledge department of biochemistry, Sri Venkateswara University, Tirupathi, India, for providing biological activity. Also, the authors express their thanks to Hyderabad Central University for providing instrumentation facilities for recording the spectra.

## REFERENCES

[1]. R. Engel, Chem. Rev. Vol. 77, pp.349, 1977.

[2]. J. Hiratake, J. Oda, *Biosci. Biotechnol. Biochem*. Vol.61, pp.211, 1997.

[3]. K.A. Schug, W. Lindner, *Chem. Rev.* Vol.105, pp.67, 2005.

[4]. K. Moonen, I. Lauryn, C.V. Stevens, *Chem. Rev*. Vol.104, pp.6177, 2004.

[5]. F. Palacios, C. Alonso, J.M. de los Santos, Curr. Org. Chem. Vol.8, pp.1481, 2004.

[6]. (a) J. A. Sikosrki, M. J. Miller, D. S. Braccolino, D. G. Cleary, S. D. Corey, J. E. Ream, D. Schnur, A. Shah, M. C. Walker, *Phosphorus, Sulfur Silicon Relat. Elem.* Vol.76, pp.375-378, 1993; (b) F. R. Atherton, C. H. Hassall, R. W. Lambert, *J. Med. Chem.* Vol.29, pp.29-40, 1986; (c) L. Maier, *Phosphorus, Sulfur Silicon Relat. Elem.* 53, pp.43–67, 1990; (d) L. Maier, H. Spoerri, *Phosphorus, Sulfur Silicon Relat. Elem.* Vol.61, pp.69-75, 1991; (e) J. Emsley, D. Hall, Chemistry of Phosphorus; Harper & Row: London, 1976. pp 494; (f) J. Z. Liu, B. A. Song, H. T. Fan, P. S. Bhadury, W. T. Wan, S. Yang, W. Xu, J. Wu, L. H. Jin, X. Wei, D. Y. Hu, S. Zeng, *Eur. J.Med. Chem.* Vol.45, pp.5108-5112, 2010; (g) J. Zhou, H. T. Fan, B. A. Song, L. H. Jin, P. S. Bhadury, D. Y. Hu, S. Yang, *Phosphorus, Sulfur, and Silicon* Vol.186, pp.81-87, 2011. (h) X. Yang, B. A. Song, L.H. Jin, X. Wei, P. S. Bhadury, X. Y. Li, S. Yang, D. Y. Hu, *Sci. China Chem.* Vol.54, pp.103-109, 2011. (i) L. Wu, B. Song, P. S. Bhadury, S. Yang, D. Hu, L. Jin, *J. Het. Chem.* Vol.48, pp.389, 2011; (j) Ch.

Subramanyam, Sk. Thaslim Basha, G. Madhava, Sk. Nayab Rasool, Sk.Adam, S. Durga Srinivasa Murthy and C. Naga Raju, *Phosphorus, Sulfur, and Silicon and the Related Elements*, Vol.192:3, pp.267-270, 2017; (k) S. Yang, X.W. Gao, C.L. Diao, B.A. Song, L. H. Jin, G. F. Xu, G.P. Zhang, W. Wang, D.Y. Hu, W. Xue, X. Zhou, P. Lu, *Chin. J. Chem.* Vol.24, pp.1581-1588, 2006; (l) E. Alonso, A. Solis, C. del Pozo, *Synlett* Vol.5, pp.698-700, 2000; (m) B. Sujatha, S. Mohan, Ch. Subramanyam, K. Prasada Rao, *Phosphorus Sulfur Silicon Relat. Elem.* Vol.192(10), pp.1110-1113, 2017; (n) D. Ravikumar, S. Mohan, Ch. Subramanyam and K. Prasada Rao, *Phosphorus, Sulfur, and Silicon and the Related Elements*, DOI: 10.1080/10426507.2018.1424163. (o) K.M. Yager, C.M. Taylor, A. B. Smith, *J. Am. Chem. Soc.* Vol.116, pp.9377-9378, 1994.

[7]. (a) G. Keglevich, E. Bálint, *Molecules* Vol.17, pp.12821-12835, 2012; (b) M. Nazish, S. Saravanan, N. H. Khan, P. Kumari, R. I. Kureshy, S. H. R. Abdi, H. C. Bajaj, *Chem Plus Chem* Vol.79, pp.1753-1760, 2014; (c) E. Balint, A. Tajti, A. Dzielak, G. Hagele, G. Keglevich, *Beilstein J. Org. Chem.* Vol.12, pp.1493-1502, 2016.

[8]. G. Keglevich, E. Bálint, R. Kangyal, M. Bálint, M. Milen, *Heteroatom Chem.* Vol.25, pp.282-289, 2014.

[9]. (a) R. Ghosh, S. Maiti, A. Chakraborty, D. K. Maiti, *J. Mol. Catal. A: Chem.* Vol.210, pp.53-57, 2004; (b) J. Tang, L. Wanga, W. Wang, L. Zhang, S. Wu, D. Mao, *J. Fluor. Chem.* Vol.132, pp.102-106, 2011; (c) H. Ghafuri, A. Rashidizadeh, H. R. E. Zand, *RSC Adv.* Vol.6, pp.16046-16056, 2016; (d) A. Wang, Y. Xu, Y. Gao, Q. Huang, X. Luo, H. An, J. Dong, *Phytochem. Rev.* Vol.14, pp.623-655, 2015; (e) S. Laschat, H. Kunz, *Synthesis* Vol.1, pp.90-95, 1992; (f) M. Tajbakhsh, A. Heydari, H. Alinezhad, *et al*. *Synthesis*, Vol.352, 2008; (g) M. M. Kabanchnik, E.V. Zobnina, I. P. Beletskaya, *Russ. J. Org. Chem.* Vol.41, pp.505-507, 2005.

[10]. (a) N. Farahani, J. Akbari, *Lett. Org. Chem.* Vol.14(7), pp.483-487, 2017; (b) M. Rostamizadeh, M. T. Maghsoodlou, N. Hazeri, Sd. M. Habibi-khorassani, L. Keishams, *Phosphorus Sulfur Silicon Relat. Elem.* Vol.186(2), pp.334-337, 2011; (c) E. Mohammadiyan, H. Ghafuri, A. Kakanejadifard, *J Chem. Sci*. 2017, doi.org/10.1007/s12039-017-1394-z; (d) S. D. Mitragotri, D. M. Pore, U. V. Desai, P. P. Wadgaonkar, *Catal. Commun.* Vol.9, pp.1822-1826, 2008; (e) S. M. Vahdat, R. Baharfar, M. Tajbakhsh, A. Heydari, S. M. Baghbanian, S. Haksar, *Tetrahedron Lett.* Vol.49, pp.6501-6504, 2008.

[11]. (a) P. Sreekanth Reddy, P. Vasu Govardhana Reddy, S. Mallikarjun Reddy, *Tetrahedron Lett.* Vol.55, pp.3336-3342, 2014; (b) J. J. Yang, N. Dang, Y.W. Chang, *Lett. Org. Chem.* Vol.6, pp.470-474, 2009.

[12]. (a) J. Lewkowski, P. Tokarz, T. Lis, K. Slepokura, *Tetrahedron* Vol.70, pp.810-816, 2014; (b) S. Motevalli, N. Iranpoor, E. Etemadi-Davan, K. R. Moghadam, *RSC Adv.* Vol.5, pp.100070-100076, 2015.

[13]. (a) C. Syama Sundar, N. Bakthavatchala Reddy, S. Sivaprasad, K. Uma Maheswara Rao, S. H. Jaya Prakash, C. Suresh Reddy, *Phosphorus, Sulfur Silicon Relat. Elem.* Vol.189, pp.551-557, 2014; (b) B. Kaboudin, F. Kazemi, N. K. Hosseini, *Res Chem Intermed* Vol.43(8), pp.4475-4486, 2017.

[14]. (a) Y.Q. Yu, D. Z. Xu, *Synthesis* Vol.47, pp.1869-1876, 2015; (b) Z. Karimi-Jaberi, Md. Amiri, *Heteroatom Chem* Vol. 21(2), pp.96-98, 2010; (c) M.M. Azaam, E. R. Kenawy, S. B. El-din, A. A. Khamis, M. A. El-Magd, *J Saudi Chem Soc* 2017, doi: 10.1016/j.jscs.2017.06.002; (d) Li. Ningbo, X. Wang, R. Qiu, X. Xu, J. Chen, X. Zhang, S. Chen, S. Yin, *Cat. Comm.* Vol.43(5), pp.184-187, 2014.

[15]. (a) A. de la Hoz, A. Draz Ortiz, A. Moreno, *Chem. Soc. Rev.* Vol.34, pp.164-178, 2005; (b) M. Hosseini Sarvari, H. Sharghi, *J. Org. Chem.* Vol.71, pp.6652-6654, 2006; (c) M. Syamala, *Org. Prep. Proced. Int.* Vol.41, pp.1-68, 2009.

[16]. K.F. Tanaka, Chem. Rev. Vol.100, pp.1025, 2000.

[17]. (a) S. U. Kazmi, S. N. Ali, S. A. Jamal, Atta-ur-Rehman, *J. Pharm. Sci.* Vol.4, pp.113-123, 1991; (b) Atta-ur-Rehman; M. Iqbal Choudhary, W.J. Thomsen, *Bioassay Techniques for Drug Development*, Harwood Academic, Publishers M. I., Amsterdam, 2001.

[18]. a). G. H. Bonjar Shahidi, "Evaluation of antibacterial properties of Iranian medicinal plants against *Micrococcus luteus, Serratia marcescens, Klebsiella pneumonia* and *Bordetella bronchoseptica*," *Asian Journal of Plant Sciences*, vol. 3, pp. 82-86, 2004. b). A.W. Bauer, M. M.Kirby, J. C. Sherris, and M. Truck, "Antibiotic susceptibility testing by a standardized single disc method," *American Journal of Clinical Pathology*, vol. 45, pp. 493-496, 1966.