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ORIGINAL PAPER

# Aluminium nitride catalyzed solvent-free synthesis of some novel biologically active α-aminophosphonates

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**Abstract** A green and atom-efficient one-pot protocol for the synthesis of some novel α-aminophosphonates using micron-particulate AlN/Al as a new reusable heterogeneous catalyst by the Kabachnik–Fields reaction under solvent-free conditions has been developed. The synthesized α-aminophosphonates were screened for several biological activities. They all were investigated to exhibit moderate to promising antioxidant, anti-inflammatory and cytotoxic properties.

**Keywords** Aluminium nitride · α-Aminophosphonates · Biological screening

## Introduction

Aminophosphonates are the immensely significant bioisosteres of amino acids displaying a broad spectrum of biological applications [1]. These are the esters of α-amino phosphoric acids, recognized as the structural analogues of corresponding α-amino acids and transition state mimics of peptide hydrolysis. This valuable class of compounds has attracted the attention of synthetic organic and medicinal chemists on account of their diverse and interesting biological and pharmacological activities such as enzyme inhibitors [2], antitumor agents [3], anti-proliferative [4], anti-HIV [5], antimicrobial [6] and antiviral [7], etc. activities. Aminophosphonates have been also extensively explored in agriculture as herbicides as well as fungicides [8]. Thus, α-aminophosphonates find wide spectrum of applications in medicinal, industrial and agricultural chemistry. Owing to their biological properties and utility as synthetic intermediates, the synthesis of α-substituted phosphonates and their functionalized derivatives is an important research objective because of their low toxicity and residual effects on mammalian. Thus, the therapeutic potential of α-aminophosphonates with improved pharmacological potency and properties prompted us to synthesize and evaluate the biological activities of some novel α-aminophosphonate derivatives.

Recently multi-component reactions (MCRs) are being extensively studied because they are efficient and versatile tools over multi-step organic synthesis for constructing highly complex molecules in a single step and thus make the synthesis simpler. Several multi-step synthetic approaches for the synthesis of α-aminophosphonates are well documented in the literature including alkylation of nucleophilic Schiff bases, Hofmann rearrangement of substituted phosphonoacetic esters, conversion of 1-hydroxyphosphonates to the corresponding 1-aminophosphonates [9], etc.

One of the approaches for the synthesis of α-aminophosphonates involves nucleophilic addition of phosphite to imine [10]. However, since many imines are hygroscopic and are not sufficiently stable for isolation; this

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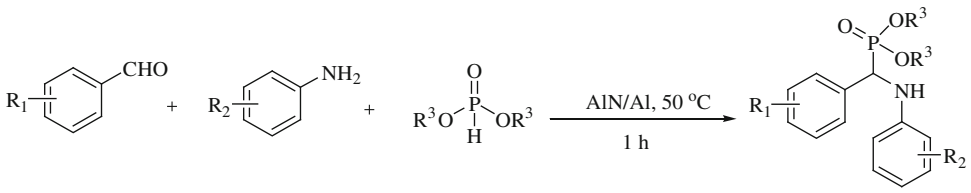



**Scheme 1** Aluminium nitride catalyzed solvent free synthesis of α-aminophosphonates

method has certain limitations. The most simple and straightforward synthetic method for the synthesis of α-aminophosphonates is the Kabachnik–Fields reaction (a MCR) which involves one-pot, three-component coupling of an aldehyde or ketone (carbonyl compound), amine and phosphite ester. In this regard, numerous protocols for the synthesis of these compounds from aldehydes, amine, dialkyl or trialkyl phosphite are developed using many Lewis and Bronsted acid catalysts such as sulfamic catalyst [11], $ZnI_2$ [12], molecular iodine [13], xanthan sulfuric acid [14], $Mg(ClO_4)_2$ [15], $FeCl_3$ [16], $Yb(PFO)_3$ [17], PEG-$SO_3H$ [18], etc. More recently, the application of microwave [19], ultrasound [20] or ionic liquid catalyzed/mediated [21] synthesis of aminophosphonates has become more popular in terms of reduced time and green nature. Research continues to develop operationally simple strategies and expeditious solvent-free synthesis of α-aminophosphonates using recyclable catalysts.

An important principle of green chemistry is replacing the use of common and hazardous organic solvents needed for chemical transformations using solvent-free protocols. The development of solvent-free multi-component methodologies for the synthesis of heterocyclic compounds has gained tremendous attention of scientific community on account of increasing hazardous effects of volatile organic solvents on the environment and thus become the intensely studied thrust area of research. Solvent-free conditions offer several distinct advantages such as clean reaction profile, enhanced reaction rates, high selectivity and higher yield.

In the current era, nano- and micron-particulate heterogeneous catalysts have emerged as sustainable alternatives rather than conventional materials due to a large surface area to volume ratio and reusable nature. The development of novel catalysts with higher catalytic activities, good recyclability and environment friendly heterogeneous nature for organic synthesis has become a significant area of research. As a part of our ongoing efforts in the development of heterocyclic compounds using reusable heterogeneous catalysts, in the present work, we wish to report aluminium nitride/aluminium as a heterogeneous catalyst for one-pot, three-component synthesis of α-aminophosphonates by the reaction of aromatic aldehyde, amine and dialkyl phosphite under solvent-free conditions at 50 °C within a short time (Scheme 1). The details of catalyst synthesis and characterization have been previously studied and reported [22].

## Experimental setup

### Synthetic method and the chemistry involved

All chemicals were purchased from SD or Aldrich chemical companies and used as such without further purification. The progress of reaction was monitored on silica gel-precoated aluminium TLC plates (Merck). Melting points were determined using capillaries open at one end and were uncorrected. $^1H$ (200 MHz) and $^{13}C$ (50 MHz) NMR spectra were recorded on a Bruker ACF 200 spectrophotometer and chemical shifts were reported on δ scale in parts per million (ppm) with the solvent indicated as the internal reference. The IR spectra were recorded on a Perkin Elmer Spectrum one-FT-IR/Bruker Vector 22 FTIR spectrophotometer using KBr discs. $^{31}P$-NMR spectra were recorded on 161.9 MHz Varian spectrophotometer with 85 % $H_3PO_4$ as external reference. The reference sample was prepared by sealing a capillary containing 85 % $H_3PO_4$ in a 5-mm NMR tube inside which is a suitable amount of DMSO-$d_6$. Mass spectra were recorded on Thermo Finnigan Surveyor MS-Q spectrometer.

### *General procedure for solvent-free synthesis of α-aminophosphonates*

In a typical reaction, aluminium nitride/aluminium catalyst (5 wt %) was added to a magnetically stirred mixture of aldehyde (2 mmol), amine (2 mmol) and dialkyl phosphite (2.2 mmol), and the contents were heated at 50 °C under stirring condition for 60 min. The progress of reaction was monitored by TLC (20 % EA:hexane). After completion of reaction as monitored by TLC, the reaction mass was diluted with methanol and filtered off to separate the catalyst. The residue being a heterogeneous material was recovered catalyst which can be recycled several times without much decrease of its catalytic activity. The filtrate was concentrated and purified by column chromatography using silica gel (100–200 mesh) as adsorbent and ethyl acetate:hexane with gradient eluent to afford pure α-amino

phosphonic acid esters as the pure products. The structures of synthesized compounds were established by $^{1}H$, $^{13}C$, $^{31}P$ NMR, IR and mass spectral data.

The spectral data of synthesized compounds are represented below:

Diethyl(4-fluorophenylamino)(phenyl) methylphosphonate ($P_1$): White solid, M.P. (°C) 132–134; $^{1}H$ NMR (200 MHz, DMSO-$d_6$) δ ppm 1.05 (3H, t), 1.20 (3H, t), 3.6–3.7 (q, 2H), 4.05 (2H, q), 5.06 (s, 1H), 6.3 (t, 1H), 6.6–7.00 (d, 4H, J = 8), 7.27–7.56 (5H, m); $^{13}C$ NMR (50 MHz, DMSO-$d_6$) δ ppm 16.41, 52.34, 55.36, 62.61, 100.37, 103.25, 109.82, 128.19, 130.27, 136.80, 149.62, 161, 165.75; IR (KBr) $cm^{-1}$ 3,285.88, 1,616.42, 1,593.27, 1,485.15, 1,231.16, 1,012.67, 955.77, 762.88; ESMS 360.02 ($M+Na^{+}$).

Diethyl(4-fluorophenylamino) (3,4-dimethoxyphenyl) methylphosphonate ($P_2$): Thick oil, $^{1}H$ NMR (200 MHz, DMSO-$d_6$) δ ppm 1.05 (t, 3H), 1.22 (t, 3H), 3.74 (s, 3H), 3.76 (s, 3H), 3.76–3.9 (m, 2H), 4.03–4.1 (m, 2H), 5.09 (s, 1H), 6.3 (t, 1H), 6.68 (3H, d, J = 8), 6.9 (1H, d, J = 8), 7.01 (2H, d, J = 8), 7.19 (1H, s); $^{13}C$ NMR (50 MHz, DMSO-$d_6$) δ ppm 16.39, 51.97, 55.73, 62.52, 100.40, 103.23, 109.95, 111.49, 112.50, 120.91, 128.93, 130.28, 148.53, 150.04, 161.02. IR (KBr) $cm^{-1}$ 3,296.49, 2,980.15, 2,933.85, 1,616.42, 1,593.27, 1,513.22, 1,232.57, 1,020.39, 951.91, 759.99, 618.21; $^{31}P$ (161.9 MHz, DMSO-$d_6$) δ ppm 22.76; ESMS: 419.97 ($M+Na^{+}$).

Diethyl(4-isopropylphenylamino)(4-methylthio)phenyl) methylphosphonate ($P_3$): Faint yellow solid, M.P. (°C) 160–162; $^{1}H$ NMR (200 MHz, $CDCl_3$) δ ppm 1.17 (d, 6H), 1.28 (t, 6H), 2.45 (s, 3H), 2.78 (m, 1H), 3.7 (d, 1H), 3.95–4.15 (q, 4H), 4.74 (s, 1H), 6.49–6.54 (d, 2H, J = 10), 6.95–6.99 (d, 2H, J = 8), 7.26 (d, 2H, J = 8), 7.41 (d, 2H, J = 8); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ ppm 16.52, 24.16, 33.12, 54.38, 57.38, 63.43, 113.86, 124.88, 126.50, 127.07, 128.37, 132.89, 138.96, 144.35; IR (KBr) $cm^{-1}$ 3,415, 3,306, 2,934, 1,614, 1,518, 1,237, 1,216, 1,053, 757; ESMS: 430.10 ($M+Na^{+}$).

Dibutyl(4-isopropylphenylamino)[4-methylthio)phenyl] methylphosphonate ($P_4$): Pale brown solid, M.P. (°C) 168–170; $^{1}H$ NMR (200 MHz, $CDCl_3$) δ ppm 0.89 (t, 6H), 1.17 (d, 6H), 1.25–1.6 (m, 8H), 2.45 (s, 3H), 2.68–2.82 (m, 1H), 3.66–3.70 (t, 1H), 3.86–4.07 (t, 4H), 4.75 (s, 1H), 6.49–6.54 (d, 2H, J = 10), 6.95–6.99 (d, 2H, J = 8), 7.22 (d, 2H, J = 8), 7.37 (d, 2H, J = 8); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ ppm 13.61, 15.72, 18.68, 24.17, 32.44, 33.12, 54.37, 57.38, 66.97, 113.85, 126.62, 127.06, 128.38, 133.04, 138.91, 144.40; IR (KBr) $cm^{-1}$ 3,307, 2,938, 1,614, 1,518, 1,234, 1,018, 824, 754; ESMS: 486.22 ($M+Na^{+}$).

Diethyl(4-isopropylphenylamino)(4-hydroxyphenyl) methylphosphonate ($P_5$): Yellow solid, M.P. (°C) 147–149; $^{1}H$ NMR (200 MHz, $CDCl_3$) δ ppm 1.17 (d, 6H), 1.27 (t, 6H), 1.87 (br, s, 1H), 2.78 (1H, m), 3.74–4.11 (m, 4H), 4.62–4.74 (d, 1H), 6.57 (d, 2H), 6.69 (d, 2H), 6.99 (d, 2H), 7.26 (d, 2H), 7.78 (s, 1H, br); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ ppm 16.38, 24.17, 33.14, 57.06, 63.64, 113.99, 116.04, 126.03, 127.07, 129, 138, 144.37, 156.66; IR (KBr) $cm^{-1}$ 3,252.9, 3,375.3, 2,961, 1,614, 1,515, 1,215, 1,024.9, 821, 751; $^{31}P$ (161.9 MHz, DMSO-$d_6$) δ ppm 23.49; ESMS: 400.03 ($M+Na^{+}$).

Diethyl(4-fluorophenylamino)(9H-fluoren-3-yl)methylphosphonate ($P_6$): Cream yellow solid, M.P. (°C) 163–165; $^{1}H$ NMR (200 MHz, DMSO-$d_6$) δ ppm 1.05 (t, 3H), 1.21 (t, 3H), 3.7 (m, 2H), 3.9 (s, 2H), 4.1 (m, 2H), 5.25 (d, 2H), 6.30 (t, 1H), 6.70 (d, 3H, J = 6), 7.05 (d, 1H, J = 8), 7.34 (dd, 2H), 7.59 (d, 2H, J = 8), 7.76 (s, 1H), 7.88 (d, 2H, J = 8); $^{13}C$ NMR (50 MHz, DMSO-$d_6$) δ ppm 16.36, 49, 53, 63.64, 100.23, 100.74, 104.06, 104.48, 109.76, 126.78, 129.23, 130.58, 131, 133.31, 135.95, 149.33, 161.37, 166.14; IR (KBr) $cm^{-1}$ 3,301.31, 1,617.38, 1,493.93, 1,227.74, 1,150.59, 1,048.36, 1,018.46, 944.20, 734.91; $^{31}P$ (161.9 MHz, DMSO-$d_6$) δ ppm 22.56; ESMS: 425.99 ($M+Na^{+}$).

Diethyl(4-fluorophenylamino)(1H-indol-3-yl) methylphosphonate ($P_7$): Light brown solid, M.P. (°C) 142–144; $^{1}H$ NMR (200 MHz, $CDCl_3$) δ ppm 1.14 (t, 6H), 3.97 (m, 4H), 4.09 (s, 1H, br, NH), 5–5.13 (d, 1H), 6.32 (d, 2H), 7.14 (dd, 2H), 7.30 (d, 2H), 7.49 (d, 2H), 7.64 (s, 1H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ ppm 16.36, 30.29, 62.63, 101.82, 110.39, 111.33, 118.49, 119.41, 122.03, 124.16, 127.17, 131.62, 135.62, 146.98, 163.25; IR (KBr) $cm^{-1}$ 3,237.66, 1,627.03, 1,509.36, 1,226.78, 1,043.53, 1,014.60, 740.70; $^{31}P$ (161.9 MHz, DMSO-$d_6$) δ ppm 25.88; ESMS: 376.96 (M+1).

## Biological activity of α-aminophosphonates

### *Antioxidant activity*

*DPPH radical scavenging assay* DPPH radical scavenging assay was carried out using reported method with slight modifications [23, 24]. 1 mL of test sample (100 μM) was added to equal quantity of 0.1 mM solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by recording the absorbance at 517 nm. Ascorbic acid (100 μM) was used as reference compound.

*Hydroxyl (OH) radical scavenging assay* The OH radical scavenging activity was demonstrated with Fenton reaction [25]. The reaction mixture contained 60 μL of $FeCl_2$ (1 mM), 90 μL of 1–10 phenanthroline (1 mM), 2.4 mL of phosphate buffer (0.2 M, pH 7.8), 150 μL of $H_2O_2$ (0.17 M) and 1.5 mL of individual sample (100 μM). The reaction was started by adding $H_2O_2$. After 5 min incubation at room temperature, the absorbance was measured at 560 nm. Ascorbic acid (100 μM) was used as the reference compound.

*Superoxide radical (SOR) scavenging assay* The SOR scavenging assay was performed using the reported method [26]. Superoxide anion radicals were generated in a non-enzymatic phenazine methosulphate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In this experiment, superoxide anion was generated in 3 mL of Tris–HCl buffer (100 mM, pH 7.4) containing 0.75 mL of NBT (300 μM), 0.75 mL of NADH (936 μM) and 0.3 mL of sample (100 μM). The reaction was initiated by adding 0.75 mL of PMS (120 μM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured using spectrophotometer. Ascorbic acid (100 μM) was used as reference compound.

*Anti-inflammatory activity*

*COX inhibition assay* The assay was performed colorimetrically using COX (human ovine) inhibitor screening assay kit [27]. Briefly, the reaction mixture contains 150 μL of assay buffer, 10 μL of heme, 10 μL of enzyme (either COX-1 or COX-2) and 10 μL of sample (100 μM). The assay utilizes the peroxidase component of COX catalytic domain. The peroxidase activity was assayed spectrophotometrically by monitoring the appearance of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. Aspirin (acetylsalicylic acid, 100 μM) was used as the standard drug. The percent COX inhibition was calculated using the following equation:

$$\text{COX inhibition activity } (\%) = \left(1 - \frac{\mathrm{T}}{\mathrm{C}}\right)100.$$

*Anticancer activity*

Human cancer cell lines: HL-60 (Leukemia), MCF-7 (Breast) and normal 293 (Kidney) were obtained from National Center for Cell Sciences, Pune (MS) India. All cell lines were propagated in a minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids and 1 mM sodium pyruvate (90 %), fetal calf serum (10 %). All cell lines were grown in a humidified incubator at 37 °C.

$$\text{Inhibitory activity } (\%) = \left(1 - \frac{\mathrm{T}}{\mathrm{C}}\right)100.$$

## Results and discussion

Initially, to optimize reaction conditions for the synthesis of α-aminophosphonates, several solvents such as DCM, THF, toluene, methanol and ethanol were tried for the reaction among benzaldehyde, 4-fluoro aniline and diethyl phosphite under different conditions using 5 wt% of the catalyst (Table 1). However, solvent-free condition was observed to be more efficient leading to higher yields than the use of common organic solvents. Also the solvent-free condition at ambient temperature (r. t.) could not lead to the completion of reaction even after 5 h. When the reaction mixture was warmed to 50 °C, a clean formation of the corresponding α-aminophosphonate was observed. However, a gradual increase in the reaction temperature to 80 °C or above could not further reduce the reaction time indicating that 50 °C was the optimum reaction temperature. Furthermore, to determine the appropriate catalyst concentration, increasing the catalyst concentration from 5 to 10 and 15 wt% could not improve the reaction conditions in terms of yield and reaction time suggesting that 5 wt% of the catalyst was sufficient for the reaction. Under these optimized conditions, all the reactions proceeded smoothly with the clean formation of products irrespective of the nature of substituents attached to the aldehydes (Table 2); however, for spectroscopic characterization and biological screening, the compounds were purified using column chromatography with EA:hexane as the eluent. Thus, clean reaction profile, reduced reaction time, higher yields, simple procedure on account of heterogeneous nature of catalyst are the advantages associated with the present protocol.

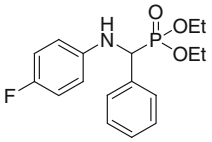
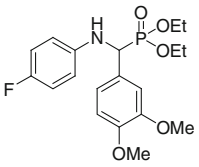
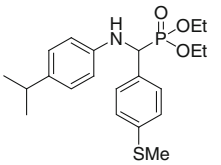
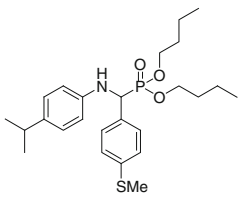
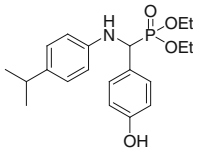
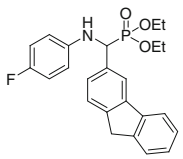
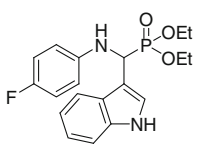
**Table 1** Optimization of reaction conditions and solvent screening

| Entry | Solvent | Temp. (°C) | Time (h) | Yield (%) |
|---|---|---|---|---|
| 1 | DCM | 40 | 4 | 38 |
| 2 | THF | 70 | 4 | 26 |
| 3 | Toluene | 100 | 4 | 53 |
| 4 | Methanol | 60 | 3 | 40 |
| 5 | Ethanol | 80 | 3 | 48 |
| 6 | Neat | r.t. | 5 | 56 |
| 7 | Neat | 50 | 1 | 94 |
| 8 | Neat | 80 | 1 | 94 |

Isolated yield of reactions on benzaldehyde (1 mmol), 4-fluoro aniline (1 mmol), diethylphosphite (1.1 mmol) in 1 mL solvent using aluminium nitride (5 wt%) of catalyst

Mechanistically, aluminium nitride functions as a mild Lewis acid which is the main active site of reaction. It facilitates the formation of imines by condensation of the carbonyl and amine functional groups. The activated imine further reacts with dialkyl phosphate leading to formation of the corresponding α-aminophosphonates. A plausible mechanism is depicted in Fig. 1. All the synthesized compounds exhibited satisfactory data. IR spectra exhibited characteristic bands at 3,200–3,400, 1,200, 1,000 $cm^{-1}$ for the stretching of N–H, P = O and P–C bonds,

**Table 2** Aluminium nitride catalyzed one-pot, three-component synthesis of α-aminophosphonates

| Entry | Aldehyde ($R_1$) | Amine ($R_2$) | Dialkyl phosphite ($R_3$) | Product | Yield (%)[a] | Melting point (°C) |
|---|---|---|---|---|---|---|
| 1 | H | 4-F | OEt |  ($P_1$) | 94 | 132–134 |
| 2 | 3,4-$(OMe)_2$ | 4-F | OEt |  ($P_2$) | 89 | Thick oil |
| 3 | 4-SMe | 4-$CHMe_2$ | OEt |  ($P_3$) | 90 | 160–162 |
| 4 | 4-SMe | 4-$CHMe_2$ | *n*-Bu |  ($P_4$) | 87 | 168–170 |
| 5 | 4-OH | 4-$CHMe_2$ | OEt |  ($P_5$) | 89 | 147–149 |
| 6 | Fluorene-3-carbaldehyde | 4-F | OEt |  ($P_6$) | 90 | 163–165 |
| 7 | Indole-3-carbaldehyde | 4-F | OEt |  ($P_7$) | 84 | 142–144 |

[a] Yields isolated after column chromatography

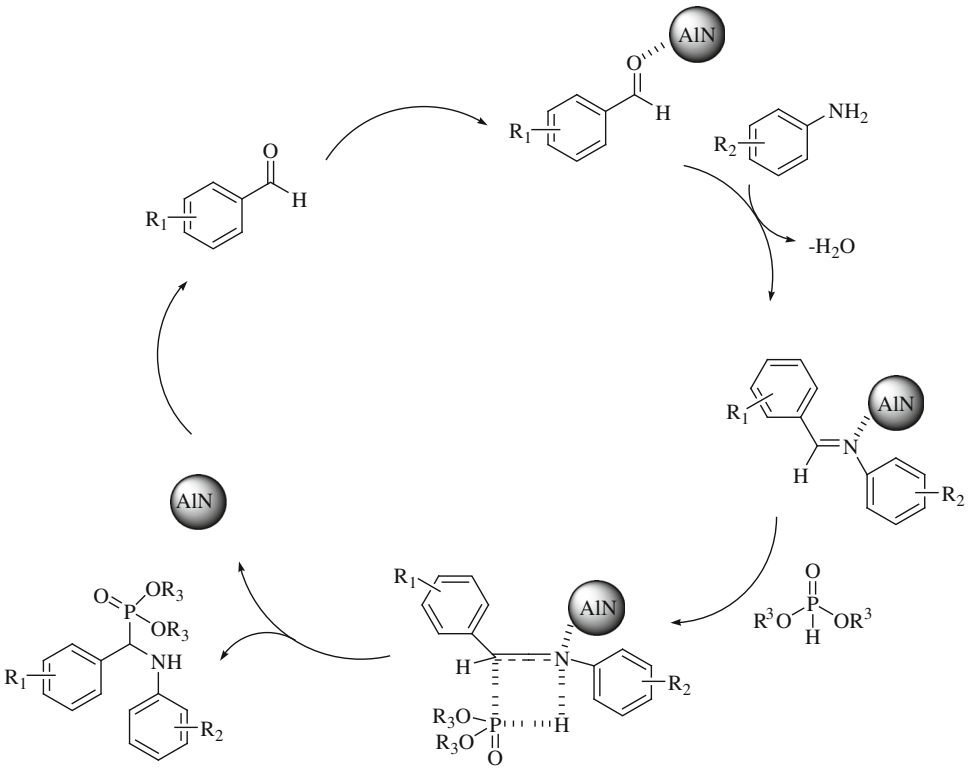



**Fig. 1** Plausible mechanism for AlN catalyzed synthesis of aminophosphonates

**Table 3** Antioxidant and anti-inflammatory activity of α-aminophosphonate derivatives ($P_1$–$P_7$)

| Entry | Compound | Radical scavenging activity (%) | | | Inhibitory activity (%) | |
|---|---|---|---|---|---|---|
| | | DPPH | OH | SOR | COX-1 | COX-2 |
| 1 | P-1 | 54.72 ± 0.25 | 63.64 ± 1.46 | 37.26 ± 1.89 | 30.18 ± 1.61 | 34.18 ± 0.96 |
| 2 | P-2 | 68.56 ± 0.75 | 50.00 ± 0.56 | 35.30 ± 0.65 | 68.51 ± 1.25 | 97.57 ± 0.47 |
| 3 | P-3 | 47.14 ± 1.23 | 31.82 ± 0.74 | 40.00 ± 1.21 | 54.78 ± 1.87 | 44.88 ± 1.65 |
| 4 | P-4 | 74.38 ± 0.69 | NR | 62.75 ± 1.47 | 89.84 ± 0.32 | 50.25 ± 1.45 |
| 5 | P-5 | 52.19 ± 0.87 | 81.82 ± 2.53 | 31.38 ± 0.56 | 51.47 ± 1.43 | 45.30 ± 1.84 |
| 6 | P-6 | 78.36 ± 1.63 | 65.91 ± 1.44 | 69.61 ± 2.03 | 78.37 ± 1.52 | 63.54 ± 1.63 |
| 7 | P-7 | 64.92 ± 1.47 | 59.10 ± 1.69 | 23.36 ± 1.78 | 81.36 ± 0.31 | 52.20 ± 0.15 |
| 8 | A.A. | 81.27 ± 0.87 | 20.63 ± 0.73 | 52.95 ± 0.83 | ND | ND |
| 9 | Aspirin | ND | ND | ND | 08.54 ± 0.37 | 11.11 ± 0.13 |

Results obtained here are $n = 3 \pm$ SD

*NR* no reaction under experimental condition, *ND* not determined, *A.A.* ascorbic acid

respectively. The aromatic protons in compounds resonated as multiplets in the region δ 6.01–7.2. P–C–H proton signal was observed to appear as a multiplet due to coupling with the magnetic nuclei P and N–H. The methyl protons of P-$OCH_2CH_3$ gave distinct triplets and the methylene protons of P-$OCH_2CH_3$ showed a multiplet, respectively [28]. The carbon chemical shifts for P–CH and P-$OCH_2CH_3$ in the title compounds were observed at around δ 43.1, 62.5 and 16.6, respectively. The $^{31}P$ NMR signals were appeared in the region δ around 22–26 for these title compounds.

## Antioxidant activity

### *Radical scavenging activity*

DPPH, hydroxyl and SOR scavenging assays were used as the parameters to investigate antioxidant properties of the title compounds (Table 3). DPPH [1,1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple colour whose intensity is measured spectrophotometrically at 517 nm. Antioxidants reduce DPPH to 1,1-diphenyl-2-picryl hydrazine, a colourless compound.

$$\underset{\text{(Purple)}}{\text{(DPPH)}} + \underset{\text{(Yellow)}}{\text{(H-A)}} \longrightarrow \text{DPPH-H} + \text{(A)}$$

Ascorbic acid shows maximum radical scavenging activity as compared to the sample compounds (P-1 to P-7) under investigation. Ascorbic acid showed 81.9 % inhibition. The other compounds showed % inhibition activity in the order of A.A. > P-6 > P-4 > P-2 > P-7 > P-1 > P-5 > P-3. From the series, P-6 shows maximum % inhibition of DPPH radical scavenging, while P-3 showed minimum % inhibition of DPPH.

*Hydroxyl radical scavenging assay*

The order of hydroxyl radical scavenging activity is AA > P-5 > P-6 > P-1 > P-7 > P-2 > P-3. The compound P-4 was non-reactive for this assay. The compound P-5 and P-6 are most reactive, while compound P-3 is found to be least reactive. This indicates that compound P-5 and P-6 are the most powerful scavengers for hydroxyl radicals, while compound P-3 has least scavenger activity.

*Superoxide radical (SOR) scavenging assay*

As per Table 3, compound P-6 showed maximum scavenging activity and P-5 and P-7 indicate least radical scavenging activity. The order of SOR scavenging activity is P-6 > P-4 > A.A. > P-3 > P-1 > P-2 > P-5 > P-7. Thus, the antioxidant activities of compound P-1 to P-7 were assayed using DPPH radical, hydroxyl radical and SOR scavenging assay. Compound P-6 possesses maximum % inhibition activity which is comparable to ascorbic acid. Compound P-3 has minimum % inhibition activity as indicated by DPPH and hydroxyl radical assay. As per SOR method, compound P-3 has moderate scavenging activity. In all, it can be concluded that compound P-6 has a maximum scavenging activity and P-3 showed minimum scavenging activity. The results of radical scavenging activity of the synthesized aminophosphonates are shown in Fig. 2.

**Table 4** Anticancer activity of synthesized α-aminophosphonate derivatives ($P_1$–$P_7$)

| Entry | Compound | Cytotoxicity (%) | | |
|---|---|---|---|---|
| | | HL-60 | MCF-7 | 293 |
| 1 | P-1 | 26.15 ± 1.65 | 27.82 ± 0.36 | 32.89 ± 1.98 |
| 2 | P-2 | 18.08 ± 1.78 | 13.39 ± 0.54 | 26.60 ± 0.32 |
| 3 | P-3 | 12.71 ± 1.69 | 06.78 ± 0.36 | 03.19 ± 1.51 |
| 4 | P-4 | 04.17 ± 1.32 | 02.96 ± 1.87 | NA |
| 5 | P-5 | 12.19 ± 0.23 | 42.17 ± 1.23 | 45.96 ± 1.63 |
| 6 | P-6 | 59.52 ± 0.59 | 47.86 ± 1.54 | 52.99 ± 1.47 |
| 7 | P-7 | 12.67 ± 0.14 | NA | NA |
| 8 | MT | 68.42 ± 1.92 | 54.29 ± 1.87 | 24.10 ± 1.69 |

Results obtained here $n = 3 \pm$ SD

*NA* no activity, *MT* methotrexate

Anti-inflammatory activity

For the study of anti-inflammatory activity, % inhibition of COX-1 and COX-2 was taken as a parameter. The order of % inhibition for COX-1 was observed as P-4 > P-7 > P-6 > P-2 > P-3 > P-5 > P-1. The order as per COX-2 was P-2 > P-6 > P-7 > P-4 > P-5 > P-3 > P-1. Aspirin showed minimum COX-1 as well as COX-2 inhibition activity. From both the above orders, it may be concluded that compound P-6 and P-7 possess good anti-inflammatory activity, while compound P-1, P-3 and P-5 possess poor anti-inflammatory activity (Fig. 3).

Anticancer activity

For the screening of anticancer activity, % cytotoxicity was calculated using HL-60, MCF-7, and 293 cell cultures. The cell line culture of HL-60 represents for leukemia. Culture MCF-7 was used as a parameter for breast cancer and 293 (Kidney) was used as a normal cell culture. Methotrexate was used as a standard anticancer agent to compare the activity of compounds under investigation (P-1 to P-6). Compound P-7 does not show cytotoxicity for MCF-7 and 293 cells. The compound P-4 does not have cytotoxicity for 293 cells Table 4. Cytotoxicity against HL-60 and MCF-7 indicates well anticancer property, whereas cytotoxicity against 293 contributes to side effect of anticancer drugs against normal cells. Compound P-6 has good anticancer property (Fig. 4). The anticancer activity of P-6 is less than the standard drug methotrexate. The compound P-6 also has maximum cytotoxicity against 293 cell culture, indicating that along with potential anticancer activity, it also possess side effects. Both anticancer and side effect potential were less than methotrexate. Compounds P-4 and

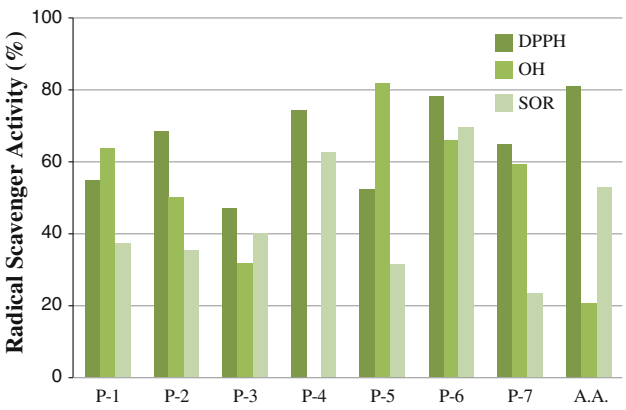



**Fig. 2** Radical scavenging activities of α-aminophosphonates

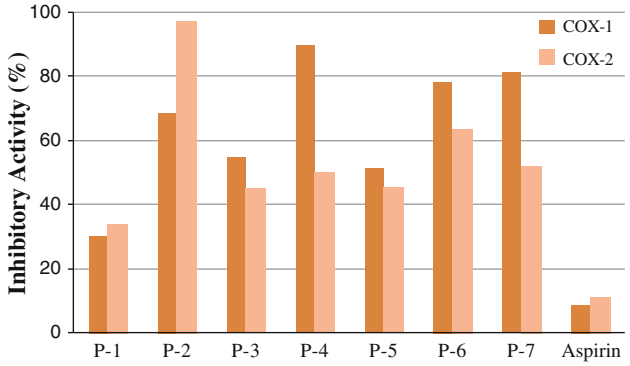



**Fig. 3** Inhibitor activities of α-aminophosphonates

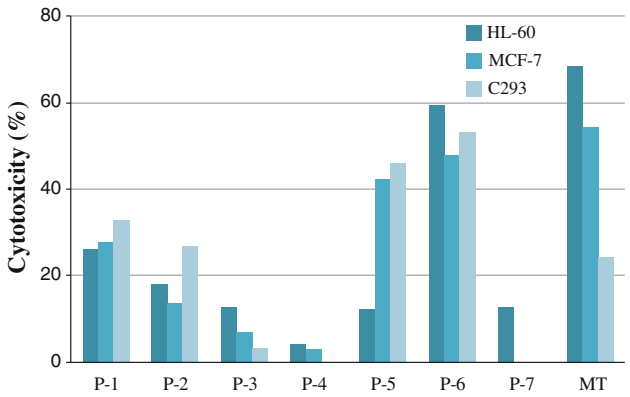



**Fig. 4** Cytotoxic studies of α-aminophosphonates

P-7 indicate less side effect as a minimal cytotoxicity for 293 cell culture, but they are least active for HL-60 and MCF-7 (compound P-7 was not effective).

## Conclusion

In summary; the present protocol provides a clean, convenient and high yielding one-pot, three-component route for the synthesis of α-aminophosphonates by the Kabachnik–Fields reaction using aluminium nitride as a new catalyst under solvent-free condition. The remarkable advantages associated with this method include solvent-free reaction condition, short reaction time, operational simplicity and high yield of products. Further studies to investigate the applications of α-aminophosphonates and biological screening of the titled compounds showed that some of these exhibit promising antioxidant, anti-inflammatory and cytotoxic activities.

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