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Ionic liquid promoted synthesis, antibacterial and in vitro antiproliferative activity of novel α-aminophosphonate derivatives

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ABSTRACT

Ionic liquid ethyl ammonium nitrate is used as an excellent catalyst and solvent for three-component one-pot reaction of an aldehydes, amines and diethylphosphite to form novel α-aminophosphonates at room temperature. Among the various catalysts, the preparation of ethyl ammonium nitrate is an environmental friendly, cost effective and recyclable catalyst. Compounds **4b**, **4c**, **4d**, 4f and **4j** were found more potent antibacterials against pathogenic microorganisms. Whereas, compounds **4a**, **4g**, 4h and **4j** inhibits growth of active *Escherichia coli* NCIM 2645 and *Salmonella typhi* NCIM 2501. Compound **4j** was found a promising antiproliferative agent against A549 and SK-MEL2 human melanoma cell lines.

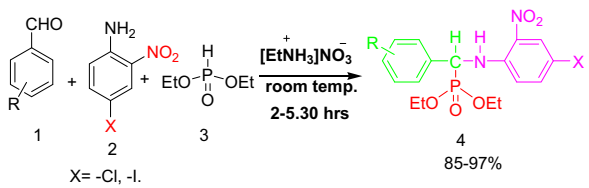
The α-aminophosphonates structural analogues of natural amino acids have received wide attention in medicinal, bioorganic and organic chemistry. The applications of α-aminophosphonates range from agriculture to medical uses as anti-cancer agents.[1] Thus, efforts are being made for the development of new methods for their synthesis.[2] There has been an increasing influence of green approach on medicinal chemistry and research based chemistry organization.[3] It is necessary to maintain greenness in synthetic pathways/processes by preventing waste generation, avoiding the use of auxiliary substances (e.g., solvents, additional reagents) and minimizing the energy requirement.[4] This needs for a development of convenient and high-yielding synthetic method.[5]

Various synthetic protocols have been described for the synthesis of α-aminophosphonates. The nucleophilic addition of phosphites to imines (Kabachnik–Fields reaction) represents a convenient route for their preparation. A variety of Lewis acids such as $SnCl_4$,[6] $ZrCl_4$,[7] $BF_3{\cdot}OEt_2$,[8] BrDMSBr (bromodimethylsulfonium bromide),[9] metal perchlorates,[10] metal triflates,[2,11] $TaCl_5$–SiO_2,[12] $InCl_3$,[13] $TiCl_4$,[14] and $SbCl_3$–Al_2O_3,[15] have been used in α-aminophosphonate synthesis. Recently anhydrous $ZrOCl_2{\cdot}8H_2O$ has been reported as an environmentally friendly catalyst for the synthesis of α-aminophosphonates.[16]

Although some significant advances have been made in reported synthesis, still it has certain limitations such as use of solvents, expensive and toxic catalyst, longer reaction time, and elevated temperature, thereby limits the applications. In many cases, two step protocols are employed, wherein a preformed imine is used.

In recent years, solvent-free reactions have gained considerable attention because these methods are not only valuable for ecological and economical reasons but also for simplicity in procedures and high yield of products. It emphasized towards the development of clean and green chemical processes and investigations of new and less hazardous catalysts.

Herein, we report ethyl ammonium nitrate (EAN) ionic liquid as an efficient and environmental friendly catalyst for the synthesis of novel α-aminophosphonates at room temperature, through three-component reaction of aromatic aldehydes, amines and diethylphosphite (Scheme 1).



Scheme 1.

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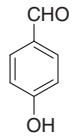
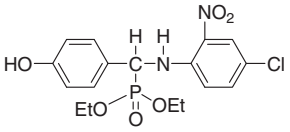
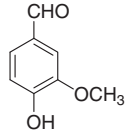
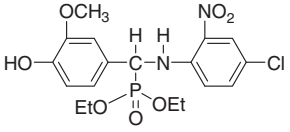
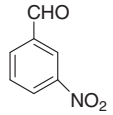
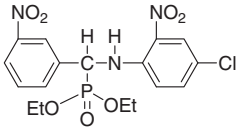
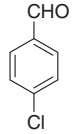
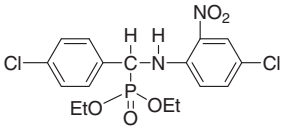
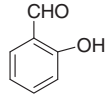
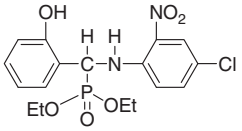
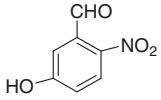
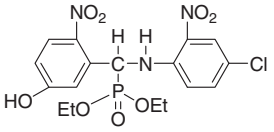
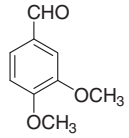
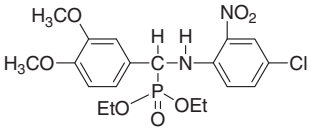
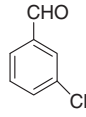
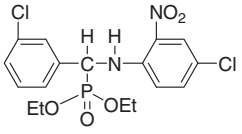
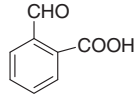
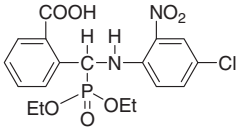
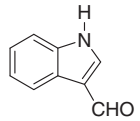
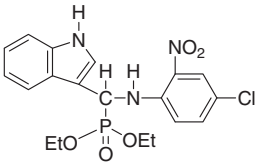
In the mid of 20th century catalysts played a vital role in synthetic chemistry. At the end of century, we saw many reforms in these fields towards clean technology and legislations. The industries are also compelled to use green technology avoiding the hazardous solvents. The green chemistry has been utilized to reduce or eliminate the use of hazardous substances. This embraces the principle of clean synthesis involving the improvements in process selectivity, high atom efficiency and easy separation with the reuse of non-product components.

Nowadays, the use of ionic liquid has been received considerable attention in the field of organic synthesis because of its mild reaction conditions, negligible vapor pressure, solvating ability and easy recyclability.[17] The efficient use of ethyl ammonium nitrate ionic liquid as catalyst can undergo a prolong way towards achieving these goals.

The ionic liquid ethyl ammonium nitrate was prepared as per literature method.[18] The ethyl ammonium nitrate was found to be a more suitable solvent for this reaction, because in presence

Table 1
Synthesis of novel α-aminophosphonate derivatives using ethyl ammonium nitrate catalyst

Entries	Reactants (**1**)	Products (**4**)	Reaction time (h)	Yields [a] (%)	Mp (°C)
a			4.00	95	141–143
b			5.00	87	132–135
c			3.00	85	107–110
d			3.30	94	114–117
e			4.00	91	125–128
f			4.30	90	119–121
g			5.00	87	105–107
h			2.00	92	120–123
i			4.45	88	232–235
j			4.30	93	170–172

[a] Isolated yield.

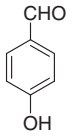
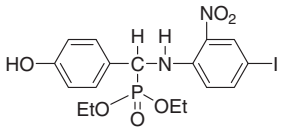
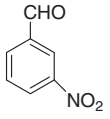
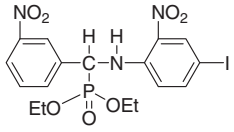
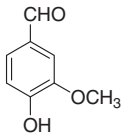
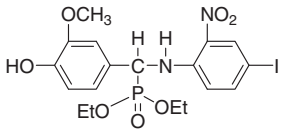
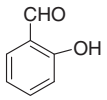
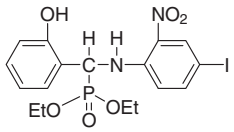
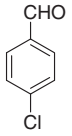
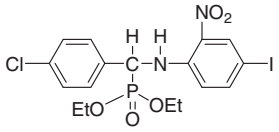
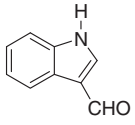
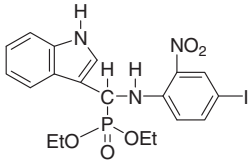
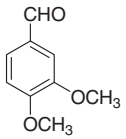
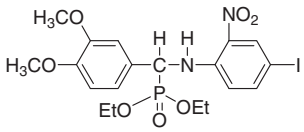
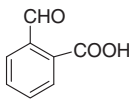
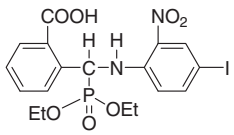
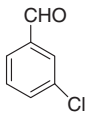
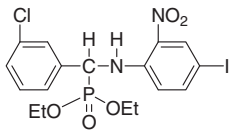
of ethyl ammonium nitrate the reaction take place in a short reaction time (2.00–5.30 h), under mild conditions and excellent yield of products 85–97% (Tables 1 and 2). In synthesis process excess of the ionic liquid was used because; ionic liquid not only acts as catalyst but also utilized as solvent to enhance the rate of reaction.

In this work, we developed new methodology for the synthesis of biologically important α-aminophosphonates using the mixture of aldehydes, amines and diethylphosphite and ethyl ammonium nitrate. The recovered ethyl ammonium nitrate was recycled and reused several times to carry out the same reaction (Table 3). Ionic liquid ethyl ammonium nitrate is water soluble. On addition of water to reaction mixture after completion of the reaction, it goes in aqueous medium which is separated by filtration and evaporation of water. It is further reused and recycled several times without any loss of its efficiency.

Ionic liquid ethyl ammonium nitrate involved in the formation of activated imines; so that the phosphite is facilitated to give a phosphonium intermediate which further reacts with water generated during imine formation to give α-amino phosphonates. In this process the neutral ethyl ammonium nitrate provide a proton for the formation of activated imine and behave as a protic solvent.

Antibacterial activity of novel α-aminophosphonate compounds: The α-aminophosphonates are found antibacterial in nature. Various food born pathogens are common agents of several diseases. Several gram negative bacteria are harmful for human beings. These bacteria develop drug resistant in a short period of time due to presence of plasmids. Antibacterial nature of nine compounds from Table 1 were analyzed against pathogenic bacteria *Escherichia coli* NCIM 2645, *Staphylococcus aureus* NCIM 2079, *Salmonella typhi* NCIM 2501, *Pseudomonas putida* NCIM 2102 and

Table 2
Synthesis of novel α-aminophosphonate derivatives using ethyl ammonium nitrate catalyst.

Entries	Reactants (**1**)	Products (**4**)	Reaction time (h)	Yields[a] (%)	Mp (°C)
a			3.00	97	175–178
b			2.30	87	108–110
c			4.30	89	105–109
d			2.00	93	162–165
e			3.00	91	168–170
f			3.30	90	205–208
g			5.30	87	197–199
h			5.00	88	221–224
i			2.30	95	135–137

[a] Isolated yield.

Table 3
Recovery of ethyl ammonium nitrate $[EtNH_3]NO_3$ in the synthesis of novel α-aminophosphonates

Entries	Products (**4**)	Percentage yield of $[EtNH_3]NO_3$		
		Recycle-1	Recycle-2	Recycle-3
1	**4a**	96	95	92
2	**4b**	98	95	93
3	**4c**	95	92	91

Proteus vulgaris NCIM 2027. These bacteria are recommended pathogenic bacteria by WHO. Out of nine compounds, that is, **4b**, **4c**, **4d**, 4f and **4j** (Table 1) were found active against all pathogens used in this study. Compound **4a** is active against *S. aureus* NCIM 2079; compounds **4g**, **4h**, 4j are inhibits growth of *E. coli* NCIM 2645 and *S. typhi* NCIM 2501. These compounds also showed antibacterial activity against *E. coli* NCIM 2645, *S. typhi* NCIM 2501. Bacterial cultures used in this experiment are resistant to Ampicillin. The compounds **4a**, 4c and **4f** inhibit growth of *S. aureus* NCIM 2079 and *P. putida* NCIM 2102. The compounds **4b**, **4c**, 4d and **4f** are the most promising and antibiotic in nature. All compounds exhibit average antibacterial activity against *P. vulgaris* 2027 except **4j** which shows very high activity. The results are also represented in Figures 1 and 2.

Antibacterial activity of compounds from Table 2: The α-aminophosphonates were used for bacterial growth inhibition. Compounds **4b** and **4h** are found to be antibacterial in nature and showed a broad range of antibacterial characters. Compounds **4e** and **4c** are most active against *P. putida* NCIM 2102 and *S. aureus* NCIM 2079. The compound **4e** inhibits the growth of *S. typhi* NCIM 2501 effectively as compare to **4h** and **4b**. Compound **4b** only inhibits the growth of *P. vulgaris* NCIM 2027.

*Antiproliferative activity of **4j***: The compound **4j** was found antiproliferative and proapoptotic in nature. The different concentrations of **4j** were used for antiproliferative assay against A549 cells and SK-MEL2, human carcinoma cells. The IC_{50} for **4j** is found 11.53 μM on A549 cells and 8.432 μM on SK-MEL2 cells. The induction of apoptosis was observed after 6 h of incubation.

Viability assay: We then examined the effect of **4j** on the viability of cultured melanoma cells by trypan blue staining. Melanoma cells were incubated with 20 μM **4j** for 24 h and their viability was assessed.

*Time dependence and anticancer specificity of **4j***: Time-course experiments using 20 μM **4j** up to 48 h shows that its anti-proliferative activity was already evident after 24 h of treatment (80–92% of growth inhibition), reaching 90–100% of growth inhibition after 36 h (Fig. 4). To simulate the activity of **4j** in vivo, we tested the response of melanoma cells to the drug after very short exposures. Indeed, wash-out experiments using 20 μM **4j** up to 48 h better defined the action time window of such compound establishing that a single administration of **4j** is sufficient to inhibit melanoma cell growth up to 80% for 24 h (Figs. 3 and 4).

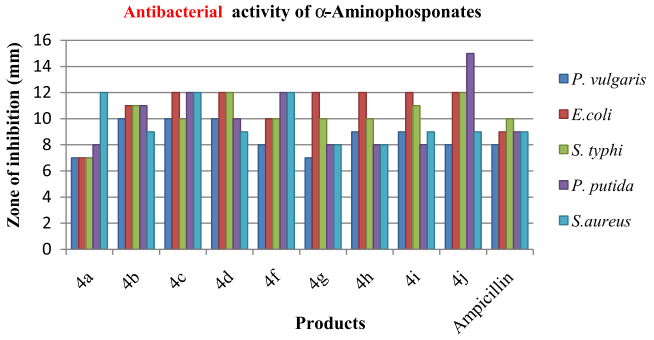


Figure 1. Antibacterial activity of α-aminophosphonates from Table 1.

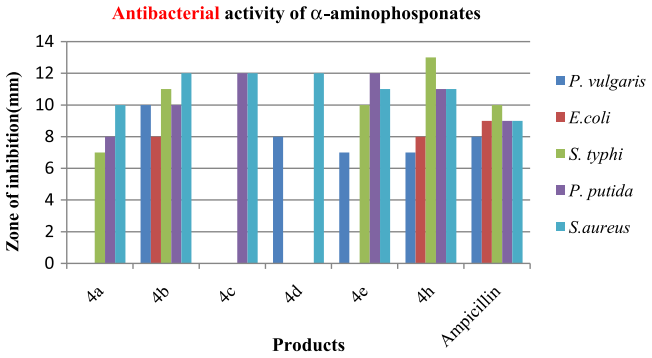


Figure 2. Antibacterial activity of α-aminophosphonates from Table 2.

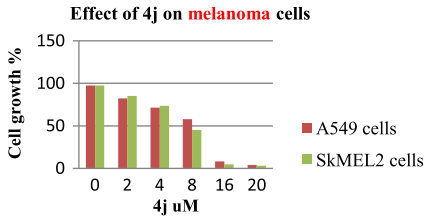


Figure 3. Effect of **4j** on the growth of human melanoma cell lines.

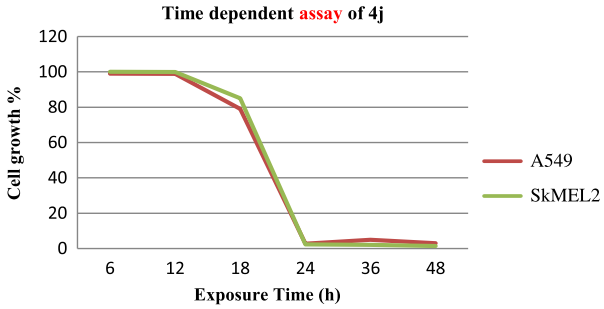


Figure 4. Time-dependent antiproliferative effect of **4j** on melanoma cells: Two melanoma cell lines were treated with 20 μM **4j** and cell proliferation was estimated at the different points, as described in Material and methods. Results are expressed as percentage of cell growth and represent the average of triplicate cultures performed twice.

Two melanoma cell lines were cultured in presence of different concentrations (0, 2, 4, 8, 16 and 20) of **4j** for 48 h and cell proliferation was estimated as described in Materials and methods. Results are expressed as percent of cell growth and represent the average of triplicate cultures performed twice.

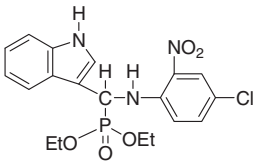
Materials and methods: *Activation of culture*: All the bacterial cultures were activated in Nutrient broth (Himedia) and incubated at respective optimum growth temperatures for 24 h.

Sample preparation: Each compound was dissolved in dimethyl sulfoxide (DMSO) to get a final concentration of 10 mg/ml and sterilized by membrane filtration.

Disc diffusion method: Discs of Whatmann filter paper No. 41 (6 mm) were prepared and autoclaved at 121 °C. Molten nutrient agar plates were seeded with 100 μl of mid log phase cultures and were allowed to solidify. Sterilized discs were placed on the surface of inoculated agar plates and 10 μl of each dissolved compound was loaded on disc. The compounds were allowed to diffuse

for 20 min. All the plates incubated at respective growth temperature. The standard antibiotic discs of Ampicillin directly placed on inoculated agar plates and incubated which would be served as positive antibacterial control. Negative control was prepared using sterile disc loaded with 10 μl of DMSO. After 24 h of incubation cultured bacteria with zone of inhibition equal to or greater than 7 mm were considered susceptible to compounds.[19]

*Antiproliferative activity of **4j**:*



4j

We used all the compounds for the in vitro antiproliferative assay. But unfortunately, only **4j** from Table 1 was found antiproliferative in nature against the melanoma cells. The other compounds showed zero/negligible antiproliferative activity (results not discussed here). Therefore we used **4j** for antiproliferative assay. The viability assay and time dependent assays were performed for the study.

A-549 and SK-MEL2 cell lines: A549 and SK-MEL2 cell lines were obtained from the National Centre for Cell Science, NCCS Pune-411007, India and seeded at a density of 10^4–10^5 cells/well on a 12-well plate Dulbecco's modified Eagle's medium (DMEM) supplemented with 5–10% FBS, 100 U/ml penicillin (or 1% penicillin–streptomycin) and 4 mM L-glutamine, at 37 °C in a humidified 5% CO_2-containing atmosphere. The plates, seeded 24 h before proceed to experiment with 10,000–100,000 cells per well, were washed 2–3 times with serum-free medium.

Viability assay: Cells were seeded at 30,000 cells/well in 12-well plates, 0, 2, 4, 8, 10, 15 and 20 μM **4j** was added to wells separately and maintained at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum, 1% non-essential amino acids. Following 24 h incubation at 37 °C viability was checked with trypan blue method.

Time dependence and sensitivity assay: The cells were seeded at 30,000 cells/well in 12-well plates. The fixed concentration of 20 μM was added to each wells and incubated for 48 h. After an interval of 6 h, the % survival of each well was calculated.

Spectral data of selected compounds: *Diethyl(4-chloro-2-nitrophenylamino)(4-hydroxy phenyl) methylphosphonate*: Table 1, entry **4a**. M.F. $C_{17}H_{20}ClN_2O_6P$; FT-IR (KBr): ν = 3355.88, 2984.04, 1613, 1467.86, 1343.89, 1245.24, 1052.65, 747.86 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.25–1.27 (t, J = 8.0 Hz, 3H), 1.33–1.31 (t, J = 3.9 Hz, 3H), 4.01 (d, 1H), 4.06–4.15 (q, J = 8.0 Hz, 4H), 4.80–4.88 (d, J = 7.9 Hz, 1H), 6.67–6.72 (dd, J = 8.0 Hz, 3H), 7.20–7.28 (m, 3H), 8.17 (s, 1H), 8.88 (br s, 1H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 157.36, 142.65, 136.22, 133.06, 128.64, 125.95, 123.86, 121.60, 116.37, 77.41, 77.08, 16.34; MS (EI, 70 eV): $[m+Na]^+$ = 437.26, m^+ = 414; ^{31}P NMR (81.01 MHz; $CDCl_3$) δ ppm: 20.21.

Diethyl(4-chloro-2-nitrophenylamino)(4-hydroxy-3-methoxyphenyl)methylphosphonate: Table 1, entry **4b**. M.F. $C_{18}H_{22}ClN_2O_7P$; FT-IR (KBr): ν = 3475.84, 3357.36, 2930.00, 1614.99, 1504.02, 1458.13, 1252.11, 1021.21, 775.25 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 1.21–1.31 (t, J = 8.0 Hz, 6H), 3.87 (s, 3H), 3.91–4.12 (q, J = 4.0 Hz, 4H), 3.9 (d, 1H), 4.80 (d, 1H), 6.57 (s, 1H), 6.67 (dd, 1H), 6.45 (dd, 1H), 7.15 (s, 1H), 7.21 (dd, 1H), 6.65 (dd, 1H), 5.09 (s, 1H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 155.22, 142.66, 136.42, 127.25, 116.22, 82.5, 77.08, 64.53.

Diethyl(4-chloro-2-nitrophenylamino)(3-nitrophenyl)methylphosphonate: Table 1, entry **4c**. M.F. $C_{17}H_{19}ClN_3O_7P$; FT-IR (KBr): ν = 3356.07, 2928.70, 1641.98, 1407.37, 1251.16, 1017.92, 818.37, 447.09 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 1.26–1.33 (t, J = 8.0 Hz, 6H), 4.06–4.19 (q, J = 5.9 Hz, 4H), 3.5–3.9 (d, 1H), 4.88–5.04 (d, 1H), 7.2–7.3 (d, 1H), 7.2 (dd, 2H), 8.23 (dd, 2H), 7.59 (s, 1H), 7.80 (s, 1H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 136.40, 126.43, 115.61, 56.68, 16.41.

Diethyl(4-chloro-2-nitrophenylamino)(4-chlorophenyl)methylphosphonate: Table 1, entry **4d**. M.F. $C_{17}H_{19}Cl_2N_2O_5P$; FT-IR (KBr): ν = 3357.09, 2975.35, 1617.46, 1408.34, 1252.37, 1056.30, 781.32, 656.07 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.24–1.33 (t, J = 8.0 Hz, 6H), 3.5–3.9 (d, 1H), 4.00–4.14 (q, J = 7.9 Hz, 4H), 4.77–4.92 (d, 1H), 8.20 (dd, 2H), 6.53–6.58 (dd, 2H), 7.24–7.38 (m, 3H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 136.23, 128.90, 115.96, 77.03, 16.44; MS (EI, 70 eV): $[m+Na]^+$ = 456.15, $[m+H]^+$ = 434.14. ^{31}P NMR (300 MHz; $CDCl_3$) δ ppm: 17.48.

Diethyl(4-chloro-2-nitrophenylamino)(2-hydroxyphenyl) methylphosphonate: Table 1, entry **4e**. M.F. $C_{17}H_{20}ClN_2O_6P$; FT-IR (KBr): ν = 3357.36, 2928.14, 1614.99, 1407.09, 1252.11, 1021.21, 816.36, 783.61, 563.25 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 1.33–1.40 (t, J = 8.0 Hz, 6H), 3.5–3.9 (d, 1H), 4.13–4.29 (q, J = 5.9 Hz, 4H), 5.40–5.56 (d, 1H), 6.66–6.85 (m, 3H), 7.01–7.27 (m, 3H), 8.16 (s, 1H), 8.93 (br s, 1H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 155.22, 136.42, 125.87, 116.22, 77.08, 16.43; ^{31}P NMR (500 MHz; $CDCl_3$) δ ppm: 21.56.

Spectral data for Table 2: *Diethyl(4-iodo-2-nitrophenylamino)(4-hydroxyphenyl)* methylphosphonate: Table 2, entry **4a**. M.F. $C_{17}H_{20}IN_2O_6P$; FT-IR (KBr): ν = 3353.67, 3100.00, 2955.00, 1608.64, 1247.69, 1018.13, 555.95 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.06–1.09 (t, *J = 8.0 Hz,* 6H), 4.07–4.10 (q, *J = 8.0 Hz,* 4H), 4.12 (dd, 1H), 4.02 (dd, 1H), 5.91 (dd, J = 8.0 Hz, 3H), 7.10–7.27 (m, 3H), 8.2 (s, 1H), 5.0 (br s, 1H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 164.71, 129.70, 127.77, 125.39, 60.61, 13.70; MS (EI, 70 eV): $[m+Na]^+$ = 529.48, $[m+H]^+$ = 506.00. ^{31}P NMR (300 MHz; $CDCl_3$) δ ppm: 18.37.

Diethyl(4-iodo-2-nitrophenylamino)(3-nitrophenyl)methylphosphonate: Table 2, entry **4b**. M.F.$C_{17}H_{19}IN_3O_7P$; FT-IR (KBr): ν = 3377.54, 2925.00, 1610.53, 1553.29, 1493.22, 1231.56, 1080, 860.12, 808.93, 693.00, 519.11 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 1.37–1.41 (t, J = 8.0 Hz, 6H), 4.21–4.29 (q, J = 7.9 Hz, 4H), 5.49–5.55 (dd, 1H), 7.06 (d, 1H), 7.21–7.24 (dd, J = 5.0 Hz, 2H), 8.18 (s, 1H), 6.71–6.73 (dd, 2H), 6.83 (s, 1H), 8.97 (br s, 1H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 155.28, 142.57, 136.33, 133.05, 129.71, 127.23, 125.85, 121.51, 120.35, 116.21, 77.05, 63.90, 16.34; MS (EI, 70 eV): $[m+K]^+$ = 553.81, m^+ = 535.23. ^{31}P NMR (300 MHz; $CDCl_3$) δ ppm: 16.50.

Diethyl(4-iodo-2-nitrophenylamino)(4-hydroxy-3-methoxyphenyl)methylphosphonate: Table 2, entry **4c**. M.F.$C_{18}H_{22}IN_2O_7P$; FT-IR (KBr): ν = 3475.84, 3357.36, 2930.00, 1614.99, 1504.02, 1458.13, 1252.11, 1021.21, 816.86, 763.61 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 1.21–1.31 (t, J = 8.0 Hz, 6H), 3.87 (s, 3H), 3.91–4.12 (q, J = 3.9 Hz, 4H), 3.9 (dd, 1H), 4.80 (dd, 1H), 6.66 (s, 1H), 6.78 (dd, 1H), 6.40 (dd, 1H), 8.17 (s, 1H), 7.21 (dd, 1H), 6.54 (dd, 1H), 5.03 (s, 1H); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 155.22, 142.66, 136.42, 125.87, 121.53, 116.22, 77.08, 64.53, 16.43; ^{31}P NMR (300 MHz; $CDCl_3$) δ ppm: 18.31.

Diethyl(4-iodo-2-nitrophenylamino)(2-hydroxyphenyl) methylphosphonate: Table 2, entry **4d**. M.F. $C_{17}H_{20}IN_2O_6P$; FT-IR (KBr): ν = 3195.41, 2928.9, 1606.88, 1460.58, 1258.65, 1025.30, 761.77, 561.07 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.27–1.3 (t, J = 8.0 Hz, 6H), 4.19–4.23 (q, J = 7.9 Hz, 4H), 4.10 (dd, 1H), 5.3 (dd, 1H), 6.46 (d, 1H), 7.2 (m, 3H), 7.55 (dd, 1H), 8.5 (s, 1H), 8.80 (m, 1H); ^{31}P NMR (500 MHz; $CDCl_3$) δ ppm: 21.08.

Diethyl(4-iodo-2-nitrophenylamino)(4-chlorophenyl) methylphosphonate: Table 2, entry **4e**. M.F. $C_{17}H_{19}ClIN_2O_5P$; FT-IR (KBr): ν = 3376.9 2927.5, 1609.62, 1494.60, 1295.29, 1249.10, 1064.80,

807.64, 546.91 cm^{-1}; ^{13}C NMR (50.32 MHz, $CDCl_3$) δ ppm: 157.34, 144.22, 134.85, 128.69, 116.42, 77.08, 64.24, 16.36; ^{31}P NMR (300 MHz; $CDCl_3$) δ ppm: 17.41.

A rapid protocol has been investigated for the synthesis of biologically active novel α-aminophosphonates using ethyl ammonium nitrate as catalyst.[20] Apart from being relatively nontoxic and environmentally friendly, the catalyst offers other advantages such as greater substrate compatibility, high reaction yields, short reaction times, recyclable, reusable and the ability to tolerate functional groups, making it an important in addition to the reported methods. Aromatic amines and aldehydes are lipophilic in nature; utilized in therapeutic applications. Hence, we used aromatic amines and aldehydes for these reactions.

Among the tested bacterial species *S. typhi* 2501, *E. coli* 2645 and *P. vulgaris* 2027 are pathogenic species belonging to *Enterobacteriaceae* family. All the novel α-aminophosphonates exhibit antibacterial activity against *P. vulgaris* 2027 except **4j** which shows very high activity against it. Compound **4j** is more potent cytotoxic agent used for antiproliferation and induction of apoptosis in A549 and SK-MEL2 cell lines. This could be a broad spectrum antibiotic and a most promising antiproliferative agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.039.

References and notes

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20. *Experimental section*: melting points were measured in open glass capillaries on a Perfit Electrothermal melting-point apparatus and are not very accurate.
Preparation of ethyl ammonium nitrate as catalyst To an aqueous solution of ethyl amine (70%, 100 ml), nitric acid (30%, 330 ml) was added drop-wise with external cooling under stirring at the temperature below 10 °C. As soon as the pH of the mixture attained the value of 7.3, the addition was stopped and the mixture was stirred further for 0.5 h. The water from a mixture was removed in a rotary evaporator in a boiling water bath at a pressure of 200 mm of Hg. Final traces of water were removed at 100 °C and 1 mmHg pressure to afford the ionic liquid in quantitative yield (170 g). The ionic liquid obtained was used as catalyst in this reaction.
General procedure for synthesis of α-aminophosphonates To a mixture of aldehyde (1 mmol), amine (1 mmol), diethylphosphite (1 mmol) and ethyl ammonium nitrate (2 ml) was added in 50 ml round bottom flask and stirred at room temperature for the appropriate time (Tables 1 and 2). The progress of reaction was monitored by thin layer chromatography. After completion of the reaction 50 ml ice cold water was added to the reaction mixture. The separated solid was filtered off, dried and purified, unreacted aromatic amines separated by column chromatography using petroleum ether/ethyl acetate (7:3 ml) as an eluent.