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A heterogeneous catalyst, SiO_2-$ZnBr_2$: An efficient neat access for α-aminophosphonates and antimicrobial activity evaluation

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Abstract. An efficient, environmentally benign and green method was developed for the synthesis of α-aminophosphonates by one-pot three-component reaction (Kabachnik-Fields reaction) of amine (4-(4-chlorophenoxy)aniline), aldehydes and diethyl phosphite using catalyst, SiO_2-$ZnBr_2$ under solvent-free conditions. The developed method under conventional conditions was further optimized in microwave and ultrasonication methods. A series of α-aminophosphonates, diethyl (4-(4-chlorophenoxy)phenylamino)(aryl/heteroaryl)methylphosphonates was synthesized to check the generality. The catalyst, SiO_2-$ZnBr_2$ afforded good yields of products in all the methods in the range of 85–97% but variation was observed in reaction time. Microwave irradiation method took very less time (4–8 min) as compared with ultrasonication (35–52 min) and conventional (2–3 h) conditions. The major advantages are simple and mild conditions, short reaction times, high yield of the product with purity, use of cheap catalyst and reusability of the catalyst until to three times without significant loss of activity. Antibacterial and antifungal activities were evaluated for the title compounds (50 and 100 μg/mL) including minimum inhibitory concentrations. A few of the newly synthesized α-aminophosphonates exhibited promising antimicrobial activity at lower MIC values in the range of 15.0–25.0 μg/mL and closer to the standards (5–12 μg/mL).

Keywords. Kabachnik-Fields reaction; α-aminophosphonates; SiO_2-$ZnBr_2$; solvent-free conditions; ultrasonication; microwave; antimicrobial activity.

1. Introduction

α-Aminophosphonates are an important class of organophosphorus compounds which find applications in numerous fields such as medicinal, bioorganic, agriculture and organic chemistry. These derivatives have received considerable attention since they are elegant peptide mimics of biologically active α-aminoacids, able to function as α-aminocarboxylic acid surrogates[1] and intriguing pharmacological and biological applications.[2] Most mammalian cell containing enzymes can hydrolyze phosphate groups, thus, phosphonates are important, stable and very low toxic unique moieties.[3] Numerous α-aminophosphonates have been reported to show broad spectrum of bioactivities such as antibiotics,[4] herbicides,[5] fungicides and plant growth regulators,[6] anti-thrombotic agents,[7] peptidases and proteinases,[8] enzyme inhibitors,[9] suppressors of the growth of various tumors and viruses.[10] Moreover, some aminophosphonic acids inhibit bone resorption, delay the progression of bone metastases and exert direct cytostatic effects on a variety of human tumor cells. Some derivatives have been found clinical applications in the treatment of bone disorder and cancer[11] and hapten design for antibody generation.[12] The stupendous applications of α-aminophosphonates have encouraged the researchers to develop new active derivatives and methods for their synthesis. We intended to synthesize, as in part of our continuing research, a library of new α-aminophosphonates, but did not achieve good yield of the products. Therefore, we developed a new synthetic protocol to accomplish high yield of α-aminophosphonates.

The classic preparation of α-aminophosphonates is generally considered to proceed *via* condensation of amine with aldehyde, consequently, addition of the phosphite to the resulting imine. One well-known approach to attain α-aminophosphonates is Kabachnik-Fields reaction.[13]

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It is a one-pot three-component reaction of amine, aldehyde and di- or trialkylphosphite and executed in the presence of different Lewis acids and Bronsted acids. Some of the variety of catalysts used are $InCl_3$,[14a] $BiCl_3$,[14b] $FeCl_3$,[14c] $YbCl_3$,[14d] $In(OTf)_3$,[14e] $Ce(OTf)_3$,[14f] $Al(OTf)_3$,[14g] $LiClO_4$,[14h] $Yb(PFO)_3$[14i] and $SbCl_3/Al_2O$,[14j] $Cd(ClO_4)_2.XH_2O$[14k] (Lewis acids), hypophosphorus acid,[15a] sulfamic acid,[15b] oxalic acid,[15c] heteropoly acids,[15d] silica-sulfuric acid,[15e] (Brønsted acids), amberlyst-15,[16] $CaCl_2$[17] and PPh_3,[18] other catalysts such as ZnO,[19] TiO_2,[20] β-cyclodextrine (β-CD),[21] NBS,[22] nano-SiO_2[23] and mesoporous aluminosilicate-nanocage.[24] In addition, methods have been developed without using any catalysts.[25] Since amines and water that are formed during imine formation can decompose or deactivate some of the catalysts, these reactions cannot be carried out in a 'one-pot' operation.

Recently, chemical transformations involving eco-friendly reagents such as solid supported catalysts have gained popularity because these methods are not only valuable for ecological and economic reasons but also for the simplicity of procedure and high yields. As well as, sonochemistry and microwave irradiated reactions are alternative methods to solve the problem of poor yields and long reaction times which can initiate some strange reactions. In the beginning, silica was originally introduces as only a support to the catalyst, but, kinetic studies revealed that it not only acts as a carrier to increase the surface area but also enhances the rate of reaction. Recently, our group have utilized some silica supported catalysts, SiO_2-$CeCl_3$[26a,b] and SiO_2-$ZnCl_2$[26c] successfully in the synthesis of phosphorus derivatives like α-aminophosphonates and carbamoyl/carbamothioyl phosphonates. Further, zinc halides, particularly a $ZnBr_2$, are of a great research interest due to their low toxicity, ease of handling, low cost, stability in air and water, recoverability and use in numerous chemical transformations.[27a–d] To the best of literature knowledge, no synthetic report is found using SiO_2-$ZnBr_2$ catalyst in the synthesis of α-aminophosphonates.

By considering the above facts, we decided to explore the possibility of implementing for one-pot three-component reaction to the preparation of α-aminophosphonate derivatives in the presence of heterogeneous catalyst, SiO_2-$ZnBr_2$ under solvent-free conditions. The reaction conditions were optimized in conventional, ultrasonicated and microwave methods. The catalyst, SiO_2-$ZnBr_2$ has been described as an effective promoting catalyst to the preparation of α-aminophosphonates under three methods and afforded high yield of the products in the range of 85–97%. The antimicrobial activity was screened for the title compounds including minimum inhibitory concentration. Some of the compounds acted as good antimicrobial agents in the range of MIC15.0–25.0 μg/mL.

2. Experimental

2.1 *Materials*

The starting material, 4-(4-chlorophenoxy)aniline and numerous aldehydes were procured from Sigma-Aldrich and used without further purification. $CDCl_3$ was purchased from Sigma-Aldrich. Reagent grade solvents, catalysts and silica gel, 100–120 mesh were acquired from Spectrochem. The instruments, BANDELIN SONOREX Model: RK 102 H for ultrasonication and CATALYST SYSTEMS CATA-4R for microwave irradiation were used. Commercial grade solvents, ethyl acetate and *n*-hexane were obtained from Merck and used in workup procedure. The reaction was monitored on Merck TLC plates using 25–60% ethyl acetate:*n*-hexane as a mobile phase. Melting points were recorded in an open capillary tube by GUNA digital melting point apparatus and are uncorrected. IR spectroscopic data were recorded on Alpha FT-IR. ^1H, ^{13}C and ^{31}P NMR spectroscopic data were recorded on a BRUKER AV 400 spectrometer, and operated at 400 MHz for ^1H, 100 MHz for ^{13}C and 161.9 MHz for ^{31}P in $CDCl_3$ solvent. Tetramethylsilane (TMS) in ^1H and ^{13}C NMR, and 85% H_3PO_4 were used as internal and external standards, respectively. ESI-mass spectral data were recorded on MLP-2103 mass spectrometer. Chemical shifts were recorded on δ scale in parts per million (ppm) and multiplicities are represented as abbreviations: s (singlet), brs (broad singlet), d (doublet), (t) triplet and m (multiplet). The numbering was assigned to the title compounds and described the spectral data accordingly.

2.2 *Conventional procedure for the synthesis of compound **5e***

The mixture of 4-(4-chlorophenoxy) aniline (**2**) (220 mg, 1.0 mmol), 3-nitrobenzaldehyde (**3e**) (155 mg, 1.03 mmol), diethyl phosphite (**4**) (0.13 mL, 1.0 mmol) and SiO_2-$ZnBr_2$ (42.75 mg, 15 mol%) were charged into a flask. The reaction mass was heated to 50°C and agitated for 2 h. The mass was cooled to room temperature (20–30°C) after completion of the reaction as checked by TLC. Ethyl acetate (10 mL) was added to the reaction content and stirred for 10 min. The catalyst, SiO_2-$ZnBr_2$ was separated by filtration as residue, washed with ethyl acetate (2 × 5 mL) and the residue was dried under

vacuum at 100°C to utilize in further studies. The combined organic layer was washed with water (10 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum at 50°C to obtain crude mass. The pure compound, diethyl (4-(4-chlorophenoxy)phenylamino)(3-nitrophenyl)methylphosphonate (**5e**) was obtained by column chromatography using 20–60% ethyl acetate:*n*-hexane as mobile phase. The same procedure was adopted to synthesize other title products.

2.3 *Ultrasonication procedure for the synthesis of compound **5e***

The mixture of 4-(4-chlorophenoxy)aniline (**2**) (220 mg, 1.0 mmol), 3-nitrobenzaldehyde (**3e**) (155 mg, 1.03 mmol), diethyl phosphite (**4**) (0.13 mL, 1.0 mmol) and SiO_2-$ZnBr_2$ (28.5 mg, 10 mol%) were taken into a open vessel. The content was heated to 50°C and agitated in ultrasonicater (35 MHz) (BANDELIN SONOREX, Model-RK 102 H, SN: 303.00051138.005) for 35 min. The completion of reaction was confirmed by TLC. Ethyl acetate (10 mL) was added to the cooled reaction mass (room temperature) and stirred for 10 min. The catalyst, SiO_2-$ZnBr_2$ was separated by filtration as residue, washed the bed with ethyl acetate (2×5 mL) and the residue was dried under vacuum at 100°C to utilize in further studies. The combined organic layer was washed with water (10 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum at 50°C to obtain crude mass. The compound, diethyl (4-(4-chlorophenoxy)phenylamino)(3-nitrophenyl)methylphosphonate (**5e**) was purified by column chromatography using 20–60% ethyl acetate:*n*-hexane as a mobile phase. The same procedure was adopted to synthesize other title products.

2.4 *Microwave irradiation procedure for the synthesis of compound **5e***

The mixture of 4-(4-chlorophenoxy)aniline (**2**) (220 mg, 1.0 mmol), 3-nitrobenzaldehyde (**3e**) (155 mg, 1.03 mmol), diethyl phosphite (**4**) (0.13 mL, 1.0 mmol) and SiO_2-$ZnBr_2$ (17.1 mg, 6 mol%) were taken into a flask. The reaction mass was irradiated with microwave radiations for 4 min using Catalyst systems (CATA-4R,), % power is 65% and 465 Watts. Ethyl acetate (10 mL) was added to the reaction mixture and stirred for 10 min, after completion of the reaction as checked by TLC. The catalyst, SiO_2-$ZnBr_2$ was separated by filtration as residue, washed with ethyl acetate (2 × 5 mL) and collected residue was dried under vacuum at 100°C to utilize in further reactions. The combined organic layer was washed with water (10 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum at 50°C to obtain crude mass. The pure compound, diethyl (4-(4-chlorophenoxy)phenylamino)(3-nitrophenyl)methyl phosphonate (**5e**) was isolated by column chromatography using 20–60% ethyl acetate:*n*-hexane as a mobile phase.

2.5 *Spectroscopic data of the title products (see more data in Supplementary Information)*

2.5a *Diethyl (2-chloro-5-nitrophenyl)(4-(4-chlorophenoxy)phenylamino)methylphosphonate **(5j)***: Off white powder, M.p.: 142–145°C. IR (cm^{-1})υ_{max}/cm^{-1}: 3204 (N-H, br, str), 2982 (C-H, str), 1521 (C=C, str), 1455 (C-H, bend), 1344 (-NO_2, str), 1323 (C-O, str), 1206 (P=O, str), 957 (C-Cl, str). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (1H, s, H-6′), 8.10 (1H, d, J = 8.4 Hz, H-4′), 7.62 (1H, d, J = 8.8 Hz, H-3′), 7.36 (2H, d, J = 8.4 Hz, H-1&5), 7.15 (2H, d, J = 7.6 Hz, H-2&4), 6.77 (2H, d, J = 8.4 Hz, H-10,12), 6.65 (2H, d, J = 7.6 Hz, H-9&13), 5.47–5.49 (1H, m, H-14), 5.31–5.39 (1H, dd, J = 16.0 Hz, 8.0 Hz, H-15), 3.93–4.13 (4H, m, H-17&19), 1.24 (3H, t, J = 6.8 Hz, H-18), 1.15 (3H, t, J = 6.8 Hz, H-20) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ156.7 (C-6), 147.1 (C-5'), 142.6 (C-1'), 140.7 (C-8), 138.2 (C-11), 136.3 (C-2'), 130.6 (C-3), 129.9 (C-3'), 129.8 (C-2&4), 123.8 (C-1&5), 123.7 (C-4'), 118.5 (C-6'), 117.6 (C-9&13), 112.1 (C-10&12), 63.9 (d, J = 6.7 Hz, C-19), 63.5 (d, J = 6.9 Hz, C-17), 52.4 (C-15), 16.3 (C-18), 16.1 (C-20) ppm. ^{31}P NMR (161.9 MHz, $CDCl_3$): δ 19.04 ppm. Mass (+ mode) m/z: 525 $(M+H)^+$, (100%), 527 $(M+H^+ +2)$, (61%).

2.6 *Antimicrobial activity*

2.6a *Antibacterial activity*: The antibacterial potency of synthesized α-aminophosphonates **5a-j** was demonstrated on the selected bacterial strains, *Staphylococcus aureus* (ATCC-19433), *Bacillus subtilis* (ATCC-23857) and *Escherichia coli* (ATCC-10148) using agar well-diffusion method. Two different concentrations, 50 and 100 μg/mL of the synthesized compounds and standard, norfloxacin were prepared in DMSO. The agar well-diffusion method[28] was applied for the determination of inhibition zone and minimum inhibitory concentration (MIC). The broth culture (0.75 mL) containing 10^6 (CFU) per mL of the test strain was mixed with nutrient agar medium (75 mL) at 45°C and poured into sterile metallic Petri plate. The medium was allowed to solidify and made wells (8 mm) with a sterile metallic borer. The test sample solutions (1 mL) were added to the respective wells and DMSO served as negative

control. Triplicate plates for each microorganism strain were prepared and incubated cultured plates aerobically at 37°C for 24 h. The inhibition zone was measured around the well to determine the activity and the average value was taken as a final result.

2.6b *Antifungal activity*: The fungal strains such as *Aspergillus niger* (MTCC-1881), *Candida albicans* (ATCC-2091) and *Aspergillus fumigates* (ATCC-9197) were selected to investigate antifungal potency of the newly synthesized α-aminophosphonates, **5a-j**. Agar disc-diffusion method[29] was used to screen the activity and the antibiotic drug; nystatin was used as standard for comparison of the activity. Two different concentrations, 50 and 100 μg/mL of the synthesized compounds and standard, nystatin were prepared in DMSO. The culture from the slant was inoculated into the Potato Dextrose broth (Hi-Media) and incubated at 37°C for 48–72 h. This culture was spread on the potato dextrose agar plate. Sterile discs about 6 mm diameter soaked into the test sample solutions and these are impregnated on the surface of the media and incubated for 48–72 h at 37 ± 3°C. The zone of inhibition around the disc was measured in millimeters. The tests were repeated three times and average value was taken as final results.

2.6c *Minimum inhibitory concentration*: The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). Micro-broth dilution method[29] was used to examine MIC of the newly synthesized compounds. To examine MICs of the test solutions, various serial concentrations 50.0, 40.0, 30.0, 25.0, 22.5, 20.0, 15.0, 12.5, 10.0, 7.5, 5.0, 2.5 μg/mL of the test solutions were prepared from the stock solution. Specifically, 0.1 mL of standardized inoculum ($1–2 \times 10^7$ CFU/mL) was added to each test tube. The bacterial tubes were incubated aerobically for 24 h at 37°C and fungal tubes were incubated for 72 h at 25°C. Control was maintained for each test sample. The lowest concentration (highest dilution) of test compound that produced no visible signs of bacterial/fungal growth (no turbidity) when compared with the control tubes were regarded as MIC.

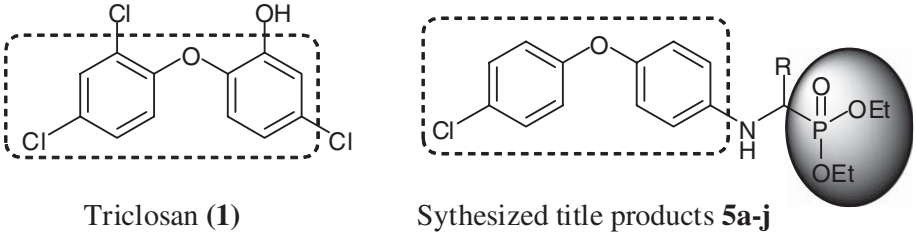
3. Results and Discussion

3.1 *Chemistry*

The promising biological activity results of α-aminophosphonates in our previous research[26a,30] encouraged us to synthesize a series of biologically active α-aminophosphonates. The amine entity, 4-(4-chlorophenoxy) aniline was chosen to prepare its α-aminophosphonate derivatives due to diphenyl ether derivatives have been potent biological activities such as antimicrobial,[31a] antitubercular,[31b] herbicidal,[31c] and serotonin transporter ligand.[31d] For example triclosan **(1)** (Figure 1) is a broad spectrum of biocide.[31e]

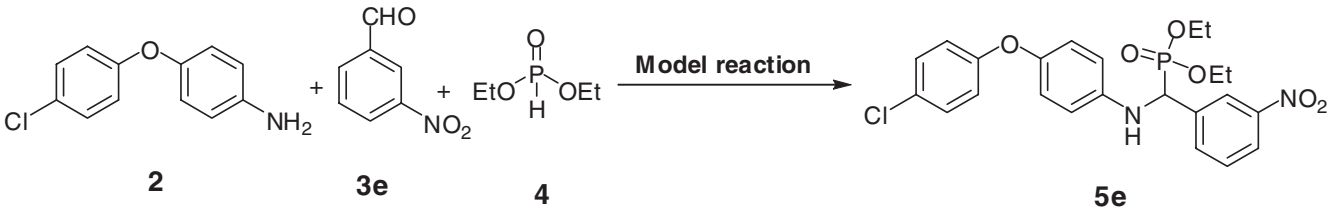
At the outset of investigation, the reaction was demonstrated with models such as 4-(4-chlorophenoxy) aniline **(2)**, 3-nitrobenzaldehyde **(3e)** and diethyl phosphite **(4)** (Scheme 1) in tetrahydrofuran (THF) solvent at reflux temperature (60–65°C), and without using any catalyst.

In this condition, the model molecules were not able to undergo effective reaction to produce the desired product, diethyl (4-(4-chlorophenoxy)phenylamino)(3-nitrophenyl)methylphosphonate **5e** and furnished only trace amount (15%) of product (Table 1, entry 1). This amount of product was not adequate for studies such as spectroscopic and biological activity evaluation; so, a new procedure was developed to acquire high yield of the product. Recent synthetic specifics of our group on the new methodologies using Lewis acid catalysts[26] have encouraged us to focus initially develop a new method, before going on to evaluation of medicinal chemistry, for achieving high yield of the α-aminophosphonates. In search for an effective catalyst and the best operational conditions, the model reaction was investigated in different Lewis acid catalysts such as $CuCl_2$, $FeCl_3$, $ZnCl_2$, $ZnBr_2$ and $CeCl_3$ (10 mol%) and the results are summarized in Table 1 (entries 2–6). It is significant to note that upon addition of Lewis acid catalysts, the yield of the product **5e** was improved from



Triclosan **(1)** Sythesized title products **5a-j**

Figure 1. Biologically active compounds.



Scheme 1. The model reaction to optimize conditions for the synthesis of compound **5e**.

Table 1. The experimental optimization results for the synthesis of product **5e**[a].

Entry	Solvent	Catalyst	Temperature	Time	Yield
1.	THF	–	65°C	18.0 h	15%
2.	THF	$CuCl_2$ (10 mol%)	65°C	10.0 h	56%
3.	THF	$FeCl_3$ (10 mol%)	65°C	6.0 h	62%
4.	THF	$ZnCl_2$ (10 mol%)	65°C	4.0 h	69%
5.	THF	$ZnBr_2$ (10 mol%)	65°C	3.5 h	74%
6.	THF	$CeCl_3$ (10 mol%)	65°C	3.5 h	75%
7.	THF	SiO_2-$ZnCl_2$ (15 mol%)	65°C	3.0 h	79%
8.	THF	SiO_2-$CeCl_3$ (15 mol%)	65°C	3.0 h	87%
9.	THF	SiO_2-$ZnBr_2$ (15 mol%)	65°C	3.0 h	85%
10.	Toluene	SiO_2-$ZnBr_2$ (15 mol%)	70°C	3.0 h	82%
11.	ACN	SiO_2-$ZnBr_2$ (15 mol%)	70°C	3.0 h	89%
12.	DCM	SiO_2-$ZnBr_2$ (15 mol%)	39°C	3.0 h	79%
13.	EtOH	SiO_2-$ZnBr_2$ (15 mol%)	65°C	3.0 h	83%
14.	1,4-Dioxane	SiO_2-$ZnBr_2$ (15 mol%)	70°C	3.0 h	85%
15.	DMF	SiO_2-$ZnBr_2$ (15 mol%)	80°C	3.0 h	82%
16.	Solvent-free	SiO_2-$ZnBr_2$ (15 mol%)	70°C	2.0 h	91%

[a]4-(4-Chlorophenoxy)aniline **(2)** (1.0 equiv), 3-nitrobenzaldehyde **(3e)** (1.03 equiv) and diethyl phosphite **(4)** (1.0 equiv.) were selected to optimize the reaction conditions under conventional method through Kabachnik-Fields reaction.

15% to the range of 54–75%, indicating that the Lewis acid catalyst playing promising role in the process. However, the catalysts, $ZnBr_2$ (74%) and $CeCl_3$ (75%) afforded high yields as compared to other catalysts. The heterogeneous (SiO_2) supported Lewis catalysts were catalyzed effectively this kind of reaction,[26] hence, the model reaction was examined in the presence of different silica supported catalysts, SiO_2-$ZnCl_2$, SiO_2-$CeCl_3$ and SiO_2-$ZnBr_2$ (15 mol%) (Table 1, entries 7–9) in THF at reflux conditions (60–65°C). The necessity to use silica supported Lewis acid catalyst was realized by the observation of high yield of the product (79–87%) when compared with the corresponding catalyst without silica support. As stated above, the catalysts, SiO_2-$CeCl_3$ (87%) and SiO_2-$ZnBr_2$ (85%) were effectively involved in the formation of the product, **5e** in high yield. In our prior synthetic specific, the method was developed for α-aminophosphonates using SiO_2-$CeCl_3$ catalyst,[26a] but till date, no report was found on SiO_2-$ZnBr_2$ in the synthesis of α-aminophosphonates. Therefore, we have selected this catalyst for further optimization studies.

The solvent effect on the reaction was also significant. So, the model reaction was scrutinized in different solvents such as toluene, acetonitrile (ACN), dichloromethane (DCM), ethanol (EtOH), 1,4-dioxane, dimethylformamide (DMF) and without solvent in the presence of SiO_2-$ZnBr_2$ (15 mol%), and the results are tabulated in Table 1 (entries 10–16). The reaction under solvent-free conditions proceeded neatly and gave high yield of the product (91%) as compared with other solvents. Substantially better conversion of the desired product **5e** (89%) was also produced in acetonitrile. The solvent-free reactions are environmentally benign and operationally simple; therefore, we have preferred neat reaction conditions.

All the reactions stated above were performed at 35–70°C and refluxing temperatures. To our delight, the model neat reaction was tested in different temperatures like RT, 40, 50 and 60°C to attain the mild condition and the results shown in Figure 2. The results revealed that no significant disparity was observed while the reactions were run in between 50–70°C; so, this range of temperature is suitable to provide the best yield of

α-aminophosphonate derivative, **5e**. The usage of catalyst amount also showed more effect in the reaction; thus, the model reaction was examined by loading different amounts of the catalyst, SiO_2-$ZnBr_2$ (Table 2, entries 1–4). As shown in Table 2, the use of catalyst less than 15 mol% amount was affected the reaction and afforded some lower yield, and did not observe significant variation when using more amount of the catalyst than that of 15 mol%. Therefore, 15 mol% amount of the catalyst was required to provide optimum yield of the product. To know the reusability of the catalyst, SiO_2-$ZnBr_2$, the product was filtered after each run and the residue of catalyst was washed with ethyl acetate to remove tar more efficiently from the catalyst surface, and then reused up to five cycles to synthesize compound **5e** which gave corresponding yields in Table 2 (entries 5–9). It was found that noteworthy deviation in yield was not observed upto three cycles of the catalyst and the catalytic activity decreased thereafter.

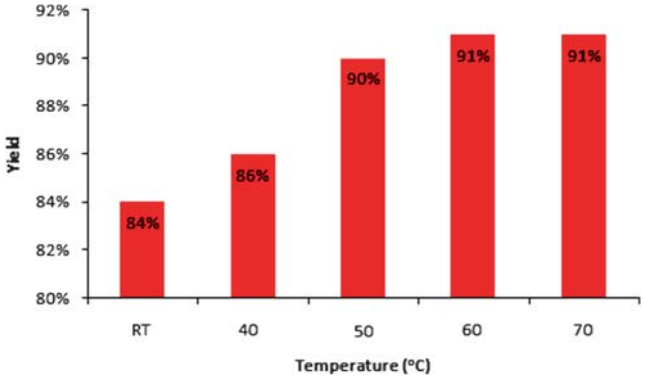


Figure 2. Temperature effect on the synthesis of compound **5e** under conventional condition: 4-(4-Chlorophenoxy) aniline **(2)** (1.0 equiv), 3-nitrobenzaldehyde **(3e)** (1.03 equiv) and diethyl phosphite **(4)** (1.0 equiv) were selected to optimize the reaction under solvent-free conditions through Kabachnik-Fields reaction.

With the optimized conditions secured, we studied the generality by altering various substituted aldehydes **3a-j** and prepared a series of α-amiophosphonate derivatives **5a-j**, and the experimental results are presented in Table 4. Excellent yields were obtained when using heteroaryl/aryl aldehydes. The reaction was compatible with various functional groups such as F, Cl, Br, OMe, NO_2 and OH that do not interfere by competitive complex formation with the catalyst. Excellent chemoselectivity was observed for substrates containing halogen atoms (**5d**, **5h**, **5i** and **5j**) that did not experience any competitive aromatic nucleophilic substitution of halogen atom. However, the reactions needed to be carried out for longer periods for aldehydes bonded with electron donating groups and lower time for electron withdrawing substituted aldehydes.

In conventional conditions, the good yields of products were obtained in the range of 83–90% but it took more time to complete the reaction. Therefore, we focused attention further to reduce the reaction time. The above stated optimized model solvent-free reaction using SiO_2-$ZnBr_2$ (15 mol%) was carried out under ultrasonication at 50°C. Interestingly, the reaction completed within 35 min and attained the compound **5e** with yield of 93% (Table 3, entry 1). Also, the effect of amount of catalyst was examined on the model reaction and the results are tabulated in Table 3 (entries 2–5). It was found that the optimum yield (92%) was obtained even using the catalyst, SiO_2-$ZnBr_2$, amount 10 mol% and upon decreasing the catalyst amount led to decreasing the product yield. Therefore, a lower amount of catalyst, 10 mol% is adequate to obtain promising yield of the product, **5e** in ultrasonication condition as

Table 2. The amount and reusability of catalyst, SiO_2-$ZnBr_2$ effect on the synthesis of **5e**[a].

Entry	Solvent	Catalyst	Temperature	Time	Yield
The loading of catalyst amount effect					
1.	No solvent	SiO_2-$ZnBr_2$ (20 mol%)	50°C	2.0 h	92%
2.	No solvent	SiO_2-$ZnBr_2$ (17.5 mol%)	50°C	2.0 h	91%
3.	No solvent	SiO_2-$ZnBr_2$ (10 mol%)	50°C	2.0 h	88%
4.	No solvent	SiO_2-$ZnBr_2$ (7.5 mol%)	50°C	2.0 h	84%
Reusability of the catalyst					
5.	No solvent	SiO_2-$ZnBr_2$ (15 mol%) (1^{st}run)	50°C	2.0 h	91%
6.	No solvent	SiO_2-$ZnBr_2$ (15 mol%) (2^{nd} run)	50°C	2.0 h	91%
7.	No solvent	SiO_2-$ZnBr_2$ (15 mol%) (3^{rd} run)	50°C	2.0 h	89%
8.	No solvent	SiO_2-$ZnBr_2$ (15 mol%) (4^{th} run)	50°C	2.0 h	85%
9.	No solvent	SiO_2-$ZnBr_2$ (15 mol%) (5^{th} run)	50°C	2.0 h	81%

[a]4-(4-Chlorophenoxy)aniline **(2)** (1.0 equiv), 3-nitrobenzaldehyde **(3e)** (1.03 equiv) and diethyl phosphite **(4)** (1.0 equiv.) were selected as models to optimize the reaction conditions under conventional method through Kabachnik-Fields reaction.

Table 3. Optimization of Kabachnik-Fields reaction under ultrasonication and microwave irradiation conditions to prepare **5e**[a].

Entries	Catalyst	Time	Yield
Ultrasoication method[b]			
1.	SiO_2-$ZnBr_2$ (15.0 mol%)	35 min	94%
2.	SiO_2-$ZnBr_2$ (12.5 mol%)	35 min	94%
3.	SiO_2-$ZnBr_2$ (10.0 mol%)	35 min	93%
4.	SiO_2-$ZnBr_2$ (8.0 mol%)	35 min	91%
5.	SiO_2-$ZnBr_2$ (5.0 mol%)	35 min	87%
Microwave irradiation method[c]			
6.	SiO_2-$ZnBr_2$ (10 mol%)	2 min	99%
7.	SiO_2-$ZnBr_2$ (9.0 mol%)	2 min	99%
8.	SiO_2-$ZnBr_2$ (8.0 mol%)	2 min	98%
9.	SiO_2-$ZnBr_2$ (7.0 mol%)	2 min	98%
10.	SiO_2-$ZnBr_2$ (6.0 mol%)	2 min	98%
11.	SiO_2-$ZnBr_2$ (5.0 mol%)	2 min	95%

[a]4-(4-Chlorophenoxy)aniline **(2)** (1.0 equiv), 3-nitrobenzaldehyde **(3e)** (1.03 equiv) and diethyl phosphite **(4)** (1.0 equiv) were selected to optimize the reaction conditions through Kabachnik-Fields reaction. [b]The reactions were maintained at 50°C in sonication conditions. [c]In microwave irradiation conditions, 65% power (465 Watts) was used.

compared with conventional condition (15 mol%). Further, the model reaction optimized under ultrasonication was also run in microwave irradiation conditions (CATA-4R, % power is 65% and 465 Watts) (Table 3, entry 6), and remarkably, a high yield **5e** (97%) was obtained in very less time (4 min). Then, the effect of amount of catalyst was investigated for 5, 6, 7, 8 and 9 mol% (Table 3 entries 7–11). The experiments revealed that almost the same yield (97%) of product **5e** was obtained when using the catalyst amount in the range of 6–10 mol% and significant lower yield (93%) for 5 mol%. Hence, optimum amount of the catalyst, 6 mol% was confirmed under microwave irradiation. To evaluate the generality, the same series of compounds **5a-j** prepared in conventional conditions was synthesized under ultrasonication and microwave irradiation conditions (Table 4). All the compounds were obtained in high yields in the range of 89–94% in the reaction time of 34–50 min under sonication conditions was 93–98% yields in the reaction time of 4–8 min under microwave irradiation. As discussed above, electron withdrawing substituted aldehydes afforded high yields as compared with electron donating substrates.

In this case, the electron withdrawing nature of oxygen atoms present in silica can increase the Lewis acid activity to the catalyst than that of without silica supporting. The Lewis acid catalyst can enrich the reactivity of aldehyde and imine intermediate by partially overlapping with heteroatoms (O and N) and catalyze the Kabachnik–Fields reaction (Figure 3). According to this mechanism, silica-supported $ZnBr_2$ catalyzed the formation of imine intermediate through generation of partial bond between the vacant site of catalyst and the oxygen atom of the carbonyl group. Further, the imine carbon can also activate, for easy attack by diethyl phosphite, by the catalyst through partial overlap with nitrogen atom to afford the desired product. The plausible mechanism of the formation of α-aminophosphonates in the presence of catalyst, SiO_2-$ZnBr_2$ is presented in Figure 3.

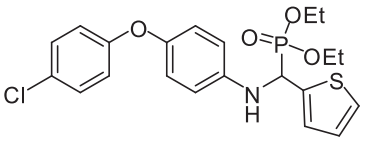
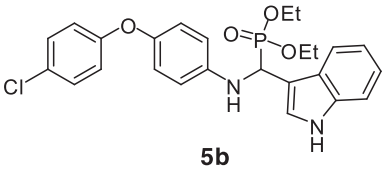
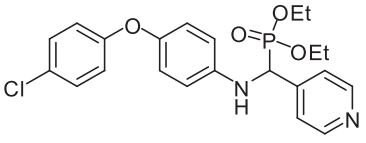
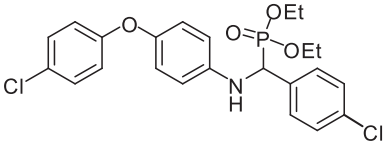
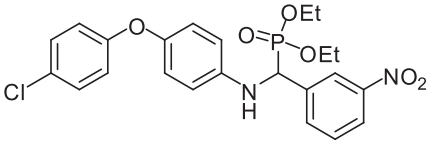
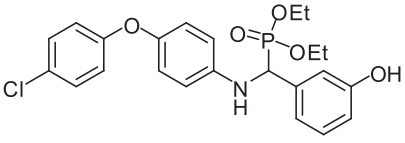
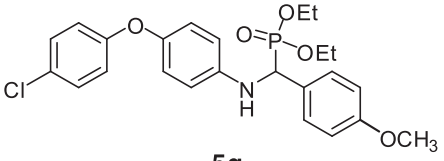
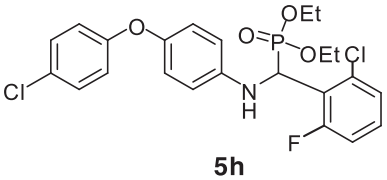
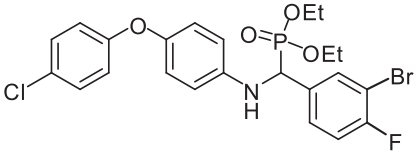
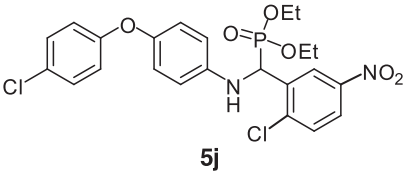
3.2 *Spectroscopic data*

Structures of the newly synthesized compounds were elucidated by spectroscopic data such as IR, NMR (^{1}H, ^{13}C and ^{31}P), ESI-MS and elemental analyses. Appearance of the absorption bands in the range of 3300–3400 cm^{-1} and 1230–1260 cm^{-1} in IR spectra are due to N-H stretching and P=O stretching, respectively. In ^{1}H NMR, the chemical shift in the range of 5.25–5.96 ppm as a doublet of doublet and 4.40–4.85 ppm as a broad singlet or multiplet were confirmed the H-15 and H-14 protons, respectively. The chemical shift values in the aliphatic region at 1.15–1.21 ppm as triplet and 3.85–4.30 ppm as multiplet are assigned to ethoxy group (–CH_3 and –O-CH_2-, respectively) in phosphonate group. Other aromatic protons are appeared in their corresponding region. In ^{13}C NMR spectra, appearance of the peaks at 16.5–18.9 ppm and 60.0–63.5 ppm confirmed the ethoxy carbons in phosphonate group, and C-15 carbon resonated at 53.0–59 ppm. The remaining aromatic and other carbons based on the structural orientation are observed in their respective regions. The phosphorus atom present in title compounds was confirmed using ^{31}P NMR spectra and showed chemical shifts in the range of 18.0–22.5 ppm. The protonated molecular mass of title compounds are observed in their corresponding ESI-MS spectra. As further evidence, C, H, N elemental analysis was obtained for a few selective compounds and the experimental composition of the title compounds are coinciding with theoretical composition.

3.3 *Antimicrobial activity*

To determine the biological potency of newly synthesized α-aminophosphonates, we screened the antimicrobial activity based on our previous specifics.[26,30] The bacterial strains, *Staphylococcus aureus* (ATCC-19433), *Bacillus subtilis* (ATCC-23857) and

Table 4. Reaction time, yield and structures of the newly synthesized title products.

 **5a**	3.0 h/86%[a] 54 min /90%[b] 6 min /92%[c]	 **5b**	2.5 h/89%[a] 45 min /91%[b] 6 min /94%[c]
 **5c**	2.0 h/92%[a] 30 min /95%[b] 4 min /98%[c]	 **5d**	2.5 h/89%[a] 40 min /90%[b] 5 min /96%[c]
 **5e**	2.0 h/91%[a] 35 min /93%[b] 4 min /98%[c]	 **5f**	3.0 h/87%[a] 50 min /88%[b] 7 min /93%[c]
 **5g**	2.5 h/88%[a] 40 min /91%[b] 6 min /96%[c]	 **5h**	3.0 h/89%[a] 46 min /90%[b] 8 min /95%[c]
 **5i**	2.5 h/90%[a] 38 min /92%[b] 5 min /98%[c]	 **5j**	2.0 h/91%[a] 38 min /94%[b] 6 min /98%[c]

[a]Time/yield of the title compounds under conventional condition. [b]Time/yield of the title compounds under ultrasonication condition. [c]Time/yield of the title compounds under microwave irradiation condition.

Escherichia coli (ATCC-10148), and fugal strains such as *Aspergillus niger* (MTCC-1881), *Candida albicans* (ATCC-2091) and *Aspergillus fumigates* (ATCC-9197) were selected to investigate the antibacterial and antifungal activities, respectively. Agar well-diffusion method[28] to examine antibacterial activity and agar disc-diffusion method[29] for antifungal activity were used. The standard drugs, norfloxacin and nystatin were used in antibacterial and antifungal activities, respectively, to compare the activity results of title compounds. The biological activity of synthesized compounds was tested at two different concentrations, 50 and 100 μg/mL in DMSO. The obtained antibacterial and antifungal activities are given in Table S1 and Table S2, in Supplementary Information, respectively.

The bio-screening data revealed that some of the compounds exhibited potent to moderate activity at 50 μg/mL and all the compounds at 100 μg/mL against both Gram positive and Gram negative bacteria, and fungi. Whereas, the compound **5b** bearing indole motif and **5i** bonded with 3-bromo-4-fluorophenyl ring exhibited potent inhibition of growth against both bacterial and fungal strains. The compounds, **5c** bonded with pyridine ring against bacterial strains, and **5d** linked with 4-chlorophenyl ring and **5g** connected with 4-methoxyphenyl ring against fungal strains showed promising activity. The derivatives, **5e** against *B. subtilis*, **5g** against *S. aureus*, and **5j** against *B subtilis* and *E. coli* exhibited potent activity. The compounds, **5c**, **5h** and **5j** exhibited potent activity against *C. albicans* and *A. fumigates*.

The minimum inhibitory concentration (MIC) of the title compounds was determined using microbroth dilution method[29] and the results are listed in Table 5. Most of the compounds showed lower minimum inhibitory concentration values in the range between 15.0–30.0 μg/mL. The derivatives, **5b**, **5c** and **5i** exhibited excellent activity at lower MIC values in

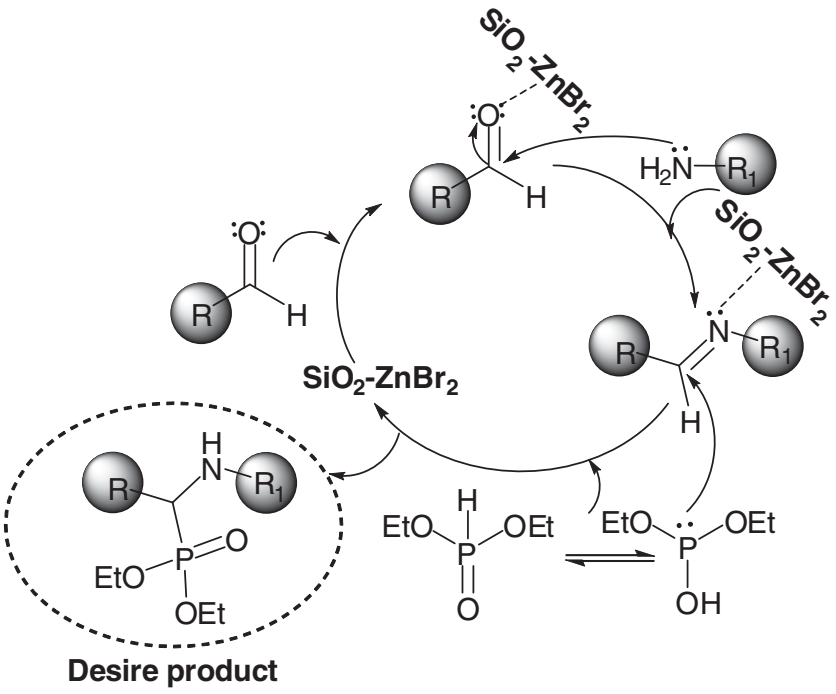


Figure 3. Plausible mechanism for the formation of α-aminophosphonates in the presence of SiO_2-$ZnBr_2$ catalyst.

Table 5. Minimum inhibitory concentration of α-aminophosphonates, **5a-j**.

	Minimum inhibitory concentration in μg/mL					
Product	*S. aureus*	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*	*A. fumigates*
5a	>40	NT	40	NT	NT	NT
5b	15.0	20.0	25.0	17.5	15.0	17.5
5c	22.5	20.0	15.0	30.0	20.0	25.0
5d	NT	NT	40	20.0	25.0	20.0
5e	NT	20.0	>30.0	NT	NT	NT
5f	NT	>40.0	30.0	NT	NT	NT
5g	22.5	30.0	NT	20.0	22.5	15.0
5h	30.0	30.0	25.0	NT	22.5	17.5
5i	17.5	22.5	15.0	20.0	17.5	15.0
5j	30.0	22.5	20.0	30.0	20.0	25.0
Std[a]	7.5	5.0	5.0	NT	NT	NT
Std[b]	NT	NT	NT	7.5	7.5	5.0

S. aureus - Staphylococcus aureus (ATCC-19433), B. subtilis - Bacillus subtilis (ATCC-23857), E. coli - Escherichia coli (ATCC-10148), A. niger - Aspergillus niger (MTCC-1881), C. albicans - Candida albicans (ATCC-2091), A. fumigates - Aspergillus fumigates (ATCC-9197). NT – Not tested.

the range of 15.0–25.0 μg/mL as compared with other compounds and these are closer to standard antibiotics (5.0–7.5 μg/mL). Compounds **5a**, **5e** and **5f** did not show MIC below 50.0 μg/mL on selected fungal strains. Some of the derivatives, **5c** against *B. subtilis*, *E. coli* and *C. albicans*, **5e** against *B. subtilis*, **5g** against *A. niger* and *A. fumigates*, **5h** against *A. fumigates*, and **5j** against *E. coli* and *C. albicans* showed MIC values below 20 μg/mL. The presence of electron withdrawing fluoro and chloro substitutions on the aromatic ring and heterocyclics such as pyridine and indole might be the causes to show promising activity as compared with other compounds.

4. Conclusions

In the present study, we have developed a green and neat one-pot three-component method using Kabachnik-Fields reaction involving aldehydes, amine, and diethyl phosphite for the synthesis of α-aminophosphonates in excellent yields in the presence of a heterogeneous catalyst, SiO_2-$ZnBr_2$ using solvent-free conditions. Three

methods such as conventional, ultrasonication and microwave irradiation were employed. The generality of new procedure was examined by altering various substituted aldehydes and prepared a series of novel diethyl (4-(4-chlorophenoxy)phenylamino)(substituted aryl/heteroaryl)methylphosphonates. Promisingly, high yields of the products were obtained in the range of 86–92% under conventional method, 89–94% under ultrasonication method and 93–98% in microwave irradiation method. However, the reaction time in microwave conditions (4–8 min) is very less compared with ultrasonication (32–52 min) and conventional (2.0–3.5 h) conditions. The described procedure has numerous benefits like avoiding harmful solvents, economical, eco-friendly, short reaction time, simple workup procedure. The heterogeneous catalyst is cheap, readily available, versatile and does not need any special precaution for preparation, handling or storage, reusable and tolerant towards various functional groups including halides, alkoxy and nitro groups. The antibacterial and antifungal activities were evaluated for the synthesized α-aminophosphonates. Whereas the compounds **5b** bearing indole motif and **5i** bonded with 3-bromo-4-fluorophenyl ring exhibited potent inhibition of growth against both bacterial and fungal strains, some of the title compounds showed promising activity against individual strains. This study is noteworthy and can provide a foundation for the design and development of some more structurally diversified α-aminophosphonates as potential antimicrobial agents.

Supplementary Information (SI)

Numbering figure of the title compounds (Figure S1), spectroscopic data of the compounds, the relevant spectra (^{1}H NMR, ^{13}C NMR, ^{31}P NMR and mass spectra) of compound **5j** (Figure S2–S5), and antibacterial (Table S1) and antifungal (Table S2) activities data are given in Supplementary Information. Supplementary Information is available at www.ias.ac.in/chemsci.

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