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Synthesis and *in-vitro* antimicrobial activity of aminomethylene bisphosphonates

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Abstract: A simple and efficient synthesis of aminomethylene bisphosphonates (N-BPs) has been developed by the reaction of diethylphosphite, triethylorthoformate and various amines in the presence of Nanoparticle TiO_2 as catalyst at 100 °C under solvent free conditions. The lead compounds were screened for their antimicrobial activity.

Keywords: N-BPs, Nanoparticle TiO_2, Diethylphosphite, Antimicrobial activity, Solvent-free conditions.

Introduction

Bisphosphonates (BPs) are having P-C-P back-bone which is structural analogues to pyrophosphates (PPs), in which the oxygen atom of P-O-P is replaced by carbon atom. Apart from their therapeutic applications, aminomethylene bisphosphonates (N-BPs) are particularly used in preventing many bone diseases such as osteoporosis, paget's, myeloma, bone metastases [1]. Further N-BPs act on bone metabolism by binding and blocking the enzyme farnesyl pyrophosphate synthase (FPPS), a key regulatory enzyme in the mevalonate pathway [2]. Apart from their biological properties, N-BPs are known for their metal chelating ability [3], and also useful as novel ligands for radioactive metal complexing that can be used in magnetic resonance imaging and imagiology, scintigraphy and radiotherapy applications [4-6].

Classical synthetic routes to aminomethylene bisphosphonates involve reductive amination of carbonyl derivatives with aminomethyl diphosphonate [7], Beckman rearrangement of oximes in the presence of phosphites [8], acid catalyzed reactions of nitriles with phosphorous acid or phosphites and condensation of amines with triethylorthoformate and phosphites [9-14]. Recently N-BPs are synthesised by using Lewis acid catalyst Amberlyst-15 [15]. However all the existing methods have drawbacks such as long reaction times, harsh reaction conditions, use of toxic catalysts and poor yields. Hence there is a need to develop a convenient, environmentally benign and practicably feasible method for the synthesis of N-BPs.

With the aim of exploiting Lewis acid-mediated synthesis of N-BPs we have successfully use the nanometal oxides composed of acidic sites, especially Lewis acidic sites. For this purpose nanocrystalline metal oxides find wide range of potential applications as active adsorbents for gases [16], oxidation of volatile organic compounds [17], the destruction of hazardous materials [18], and catalysts for many organic transformations [19-24]. Their high reactivities are attributed to high surface areas combined with unusually reactive morphologies [25].

Titanium dioxide is one such most efficient Lewis acid material for different kinds of industrial applications including selective reduction of NO in the stationary sources [26], sensors [27], food additives [28], photo catalyst for pollutant elimination [29, 30], in photo voltaic devices [31], cosmetics [32], and even in cancer treatment [33]. Further these heterogeneous catalysts are eco-friendly, easy to separate and can be reuse. These characteristics are very useful for

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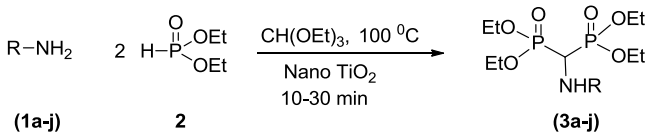
industrial process interms of economy and green chemistry.

In continuation of our interest to develop efficient synthetic routes for biologically potent organophosphorus compounds using green chemical techniques [34], the role of transition metal nanoparticles as catalysts in organic reactions [35, 36], has been explored. We now report successful synthesis of aminomethylene bisphosphonates using Nanoparticle TiO_2 as a heterogeneous catalyst. This catalyst is inexpensive, easy to handle and does not require the maintenance of anhydrous conditions. No report exists as on today on the synthesis of aminomethylene bisphosphonates using nanoparticle TiO_2 as catalyst.

Results and discussion

An efficient and environmentally benign one-pot method was developed for the synthesis of aminomethylene bisphosphonates (**3a-j**) by reaction of aromatic amines, diethyl phosphite and triethylorthoformate in the presence of nanoparticle TiO_2 (10 mol%) as catalyst at 100 °C (Scheme **1**).



Scheme 1: Synthesis of aminomethylene bisphosphonates **(3a-j).**

In order to standardise the operating reaction conditions, the reaction among benzthiazolamine, diethyl phosphite and triethylorthoformate was run as a model. At room temperature there is no sufficient quantity formation of the corresponding aminomethylene bisphosphonates in the presence of nanoparticle TiO_2 (10 mol%) catalyst even after 6 h of reaction time under solvent-free conditions (Table **1**, entry 1). Increase of the reaction temperature from 40 to 100 ºC led to formation of aminomethylene bisphosphonates up to 95 % yield. Further increase in the temperature did not show any improvement in the yield. No formation of product was observed in the absence of nanoparticle TiO_2 even after 6 h of the reaction time (Table **1**, entry 6). This shows that nanoparticle TiO_2 is absolutely essential to drive the reaction for completion. The yield was greatly affected by the amount of catalyst loaded. When 5, 10, 15 and 20 mol% of the catalyst was used, the yields varied from 90, 95, 84 and 82% respectively (Table **1**). Therefore, 10 mol% of nanoparticle TiO_2 was sufficient and use of excessive catalyst had no impact either on the rate of the reaction or on the compound yield. The reusability of the nanoparticle TiO_2 as a catalyst was also examined. After each run, the product was filtered and the catalyst residue was washed with CH_2Cl_2, dried and reused. It was found that the catalyst can be used for three cycles in the synthesis of compound 3a and in each case it gave 95%, 95%, 94% yield of the product respectively (Table **1**, entry 4).

Table 1: Optimization of reaction conditions for synthesis of 3a.

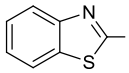
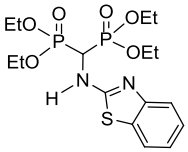
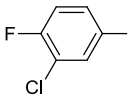
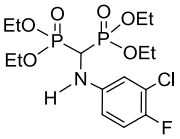
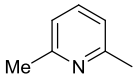
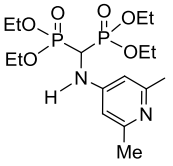
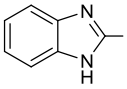
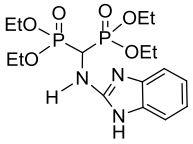
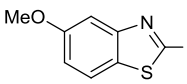
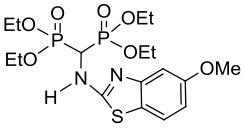
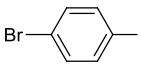
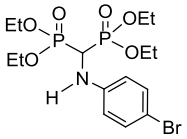
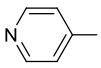
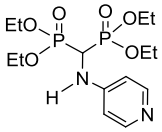
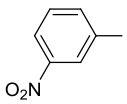
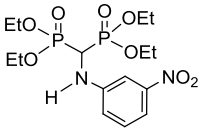
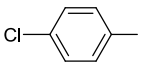
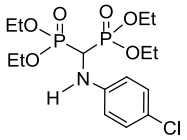
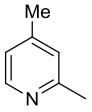
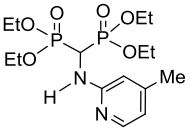
Entry	Temperature (ºC)	Nano TiO_2 (mol %)	Time (min/h)	Yield (%)[a]
1	40	10	6 h	40
2	60	10	15 min	55
3	80	10	10 min	78
4[b]	100	10	10 min	95,95,94
5	120	10	10 min	95
6	40	0	6 h	--
7	100	5	30 min	90
8	100	15	30 min	84
9	100	20	30 min	82

[a]Isolated yield
[b]Catalyst was reused three times

After optimization of experimental conditions with aniline, we extended this coupling reaction with several other amines keeping diethyl phosphite and triethylorthoformate as constant substrates and nanoparticle TiO_2 as the catalyst. In all the cases, the reactions were completed within 10-30 min and gave the corresponding aminomethylene bisphosphonates in 84-95 % yield (Table **2**). Wide applicability of this method is evident from the fact that it is tolerant towards various substrates functionalised with nitro, alkoxy and halo groups. All the title compounds **3a-j** exhibits moderate to good antibacterial and antifungal activity.

The possible mechanism for the reaction of an amine with diethyl phosphite and triethylorthoformate conducted in the presence of nanoparticle TiO_2 catalyst is presented in (Scheme **2**). The key step is the nucleophilic addition of an amine to triethylorthoformate followed by the addition of a phosphite to the resulting imine.

Table 2: Nanoparticle TiO_2 catalysed synthesis of aminomethylene bisphosphonates.

Entry	R (1)	Product (3)	Time (min)	Yield (%)[a]
a			10	95
b			20	90
c			15	85
d			25	88
e			15	90
f			20	89
g			25	86
h			20	84
i			15	85
j			30	89

[a]Isolated Yield

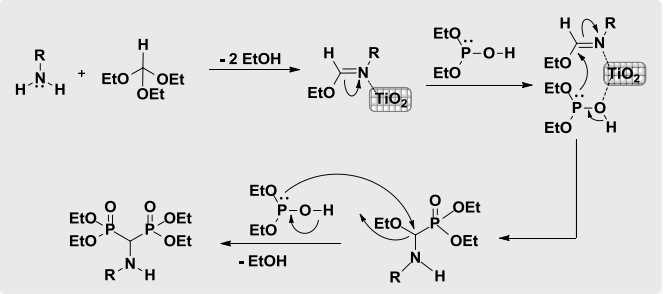
Conclusion

The present procedure using nanoparticle TiO_2 as catalyst provides an efficient synthesis of N-BPs in good to moderate yields from the direct reaction of amine, diethyl phosphite and triethylorthoformate. The main advantages of this synthetic protocol are operational simplicity, mild, eco-friendly reaction conditions, efficient and reusable catalyst, short

reaction times and good product yields. These factors make it an attractive method for aminomethylene bisphosphonates and thus serve as useful contribution to the present methodologies.

Experimental

Melting points were recorded on Buchi R-535 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-c spectrophotometer using KBr optics. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on AMX 400 MHz NMR spectrometers operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 161.7 MHz for ^{31}P NMR. NMR data were recorded in $CDCl_3$ and referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P). Mass spectra were recorded on a Finnigan MAT 1020 / Micro-Mass Q-T of micro AMPS MAX 10/6A, Hz 60/50 system fitted with a built-in inlet system. Elemental analyses were performed using Perkin Elmer 2400 instrument at the Central Drug Research Institute (CDRI), Lucknow, India.



Scheme 2: Mechanism of the aminomethylene bisphosphonates.

Procedure for the preparation of Tetraethyl (benzo[d]thiazol-2-ylamino) methylene bisphosphonate ***(3a):***

Nano crystalline TiO_2 (10 mol %) was added to a mixture of amine (0.005 mol), diethyl phosphite (0.01 mol) and triethylorthoformate (0.01 mol) and the mixture was kept stirred magnetically at 100 ºC for 10-30 minutes. After completion of reaction as indicated by TLC, the catalyst was recovered by centrifugation and washed with ether. The excess triethylorthoformate was removed in a rotary evaporator and the crude residual product was washed repeatedly with petroleum ether and water. It was purified by column chromatography on 60-120 mesh silica gel using EtOAc/n-hexane (1:4) as eluent. The product obtained was further purified by recrystallization from EtOAc to afford pure **3a** in 95% yield (Scheme **1**).

This procedure was applied successfully for the preparation of **3b-j**. All the compounds were characterized by IR, ^{1}H, ^{13}C, ^{31}P NMR, mass spectral and elemental analytical data.

Spectral data for compounds (***3a-j***):

Tetraethyl (benzo[d]thiazol-2-ylamino)methylene bisphosphonate (***3a***):

White solid, yield 95%, m.p: 150-152 °C. IR (KBr)(ν_{max} cm^{-1}); 3390 (NH), 1244 (P=O), 842 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.54-7.58 (4H, m, Ar-H), 5.95 (1H, s, NH), 4.82-5.08 (1H, m, PC<u>H</u>), 3.58-4.20 (8H, m, POC<u>H</u>$_2$CH$_3$), 1.28 (6H, t, $^{3}J_{PH}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.08 (6H, t, $^{3}J_{P-H}$=8.2 Hz, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 175.6, 157.6, 131.6, 121.5, 120, 117.6, 114.5, 63.2 (d, $^{2}J_{P-C}$=7.6 Hz, PO<u>C</u>H$_2$CH$_3$), 56.7 (d, $^{1}J_{P-C}$ =162.4 Hz, P-C-H), 17.4 (d, $^{3}J_{P-C}$ =5.6 Hz, POCH$_2$<u>C</u>H$_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 24.5. ESI-MS:(m/z) 436 (100, M^+). Anal.calcd. for $C_{16}H_{26}N_2O_6P_2S$: C, 44.04; H, 6.01. Found: C, 44.00; H, 5.95.

Tetraethyl (3-chloro-4-fluorophenylamino)methylene bisphosphonate (***3b***):

White solid, yield 90%, m.p: 120-122 °C. IR (KBr)(ν_{max} cm^{-1}); 3200 (NH), 1240 (P=O), 760 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.56-7.38 (3H, m, Ar-H), 5.90 (1H, s, NH), 4.80-5.04 (1H, m, PC<u>H</u>), 3.70-3.89 (8H, m, POC<u>H</u>$_2$CH$_3$), 1.20 (6H, t, $^{3}J_{P-H}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.06 (6H, t, $^{3}J_{P-H}$ =8.2 Hz, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 150.2, 145.2, 124.2, 120, 119.5, 115.2, 62.5 (d, $^{2}J_{P-C}$ =7.6 Hz, PO<u>C</u>H$_2$CH$_3$), 58.5 (d, $^{1}J_{P-C}$ =162.4 Hz, P-C-H), 16.5 (d, $^{3}J_{P-C}$ =5.6 Hz, POCH$_2$<u>C</u>H$_3$).^{31}P NMR (161.7MHz, $CDCl_3$): 21.5. ESI-MS:(m/z) 431 (100, M^+). Anal.calcd. for $C_{15}H_{25}ClFNO_6P_2$: C, 41.73; H, 5.84. Found: C, 41.69; H, 5.80.

Tetraethyl (6-methylpyridin-2-ylamino)methylene bisphosphonate (***3c***)**:**

White solid, yield 85%, m.p: 142-144 °C. IR (KBr)(ν_{max} cm^{-1}); 3252 (NH), 1235 (P=O), 770 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.95-7.55 (3H, m, Ar-H), 5.00 (1H, s, NH), 4.95-5.20 (1H, m, PC<u>H</u>), 3.80-4.10 (8H, m, POC<u>H</u>$_2$CH$_3$), 2.40 (3H, s, Ar-CH$_3$), 1.20 (6H, t, $^{3}J_{P-H}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.10 (6H, t, $^{3}J_{P-H}$ =8.9 Hz, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 159.2, 156.7, 150.7, 131.6, 117.6, 110.3, 107.2, 63.5 (d, $^{2}J_{P-C}$ =7.6 Hz, PO<u>C</u>H$_2$CH$_3$), 54.9 (d, $^{1}J_{P-C}$ =163.4 Hz, P-C-H), 22.5 (Ar-CH$_3$), 16.5 (d, $^{3}J_{P-C}$ =5.8 Hz, POCH$_2$<u>C</u>H$_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 18.5.

ESI-MS:(m/z) 394 (100, M^+). Anal.calcd. for $C_{15}H_{28}N_2O_6P_2$: C, 45.69; H, 7.16. Found: C, 44.68; H, 6.98.

Tetraethyl (1H-benzo[d]imidazol-2-ylamino) methylene bisphosphonate (***3d***)**:**

White solid, yield 88%, m.p: 132-134 °C. IR (KBr)(ν_{max} cm^{-1}); 3250 (NH), 1242 (P=O), 820 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.07-7.82 (4H, m, Ar-H), 6.25(1H, s, imidazole-NH), 4.95-5.20 (1H, m, PC<u>H</u>), 4.07-4.00 (8H, m, POC<u>H</u>$_2$CH$_3$), 3.63 (1H, s, NH), 1.20 (6H, t, $^3J_{PH}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.10 (6H, t, $^3J_{PH}$ =8.9 Hz, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 150.5, 140.2, 138.2, 128.5, 125.4, 122.2, 120.5, 62.5 (d, $^2J_{P-C}$ =7.6 Hz, PO<u>C</u>H$_2$CH$_3$), 56.5 (d,$^1J_{P-C}$ =163.4 Hz, P-C-H), 16.5 (d, $^3J_{P-C}$ =5.8 Hz, POCH$_2$<u>C</u>H$_3$).^{31}P NMR (161.7MHz, $CDCl_3$): 22.5. ESI-MS:(m/z) 419 (100, M^+). Anal.calcd. for $C_{16}H_{27}N_3O_6P_2$: C, 45.83; H, 6.49. Found: C, 44.98; H, 5.95.

Tetraethyl (5-methoxybenzo[d]thiazol-2-ylamino) methylene bisphosphonate (***3e***)**:**

White solid, yield 90%, m.p: 136-138 °C. IR (KBr)(ν_{max} cm^{-1}); 3350 (NH), 1210 (P=O), 760 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.89-7.64 (3H, m, Ar-H), 6.07 (1H, s, NH), 4.00-4.07 (8H, m, POC<u>H</u>$_2$CH$_3$), 3.67-3.91 (1H, m, P-C-H), 3.54 (3H, s, Ar-OCH$_3$), 1.22 (6H, t, $^3J_{P-H}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.04 (6H, t, $^3J_{P-H}$ =8.2 Hz, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 170.2, 162.5, 149.5, 125.5, 123.2, 116.5, 110.2, 62.5 (d, $^2J_{P-C}$ =7.6 Hz, PO<u>C</u>H$_2$CH$_3$), 60.2, 58.5 (d, $^1J_{P-C}$ =162.4 Hz, P-C-H), 16.2 (d, $^3J_{P-C}$ =5.8 Hz, POCH$_2$<u>C</u>H$_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 24.5. ESI-MS: (m/z) 466 (100, M^+). Anal.calcd. for $C_{17}H_{28}N_2O_7P_2S$: C, 43.78; H, 6.05. Found: C, 44.45; H, 5.89.

Tetraethyl (4-bromophenylamino)methylene bisphosphonate (***3f***)**:**

White solid, yield 89%, m.p: 140-142 °C. IR (KBr)(ν_{max} cm^{-1}); 3340 (NH), 1220 (P=O), 780 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.85-7.19 (4H, m, Ar-H), 5.85 (1H, s, NH), 3.98-4.10 (8H, m, POC<u>H</u>$_2$CH$_3$), 3.67-3.81 (1H, m, PCH), 1.26 (6H, t, $^3J_{P-H}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.02 (6H, t, $^3J_{P-H}$ =8.2 Hz, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 152.0, 129.7, 123.1, 111.3, 77.4 (d, $^1J_{P-C}$=162.4 Hz, PCH), 60.4 (d, $^2J_{P-C}$ =7.6 Hz, PO<u>C</u>H$_2$CH$_3$), 16.7 (d, $^3J_{P-C}$ =5.6 Hz, POCH$_2$<u>C</u>H$_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 21.5. ESI-MS: (m/z) 458 (100, M^+). Anal.calcd. for $C_{15}H_{26}BrNO_6P_2$: C, 39.32; H, 5.72. Found: C, 38.20; H, 5.42.

Tetraethyl (pyridin-4-ylamino)methylene bisphosphonate (***3g***)**:**

Colourless oil, yield 86%, IR (KBr) (ν_{max} cm^{-1}); 3230 (NH), 1240 (P=O), 760 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.98-7.99 (4H, m, Ar-H), 4.65 (1H, s, NH), 3.89-4.09 (8H, m, POC<u>H</u>$_2$CH$_3$), 3.65-3.79 (1H, m, P-C-H), 1.32 (6H, t, $^3J_{P-H}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.08 (6H, t, $^3J_{P-H}$ =8.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.32-1.29 (12H, m, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 154.0, 150.2, 108.5, 69.8 (d, $^1J_{P-C}$ =162.4 Hz, PCH), 61.2 (d, $^2J_{P-C}$ =7.6 Hz, PO<u>C</u>H$_2$CH$_3$), 16.5 (d, $^3J_{P-C}$ =5.6 Hz, POCH$_2$<u>C</u>H$_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 22.5. ESI-MS:(m/z) 380 (100, M^+). Anal.calcd. for $C_{14}H_{26}N_2O_6P_2$: C, 44.21; H, 6.89. Found: C, 42.02; H, 6.01.

Tetraethyl (3-nitrophenylamino)methylene bisphosphonate (***3h***)**:**

White solid, yield 84%, m.p: 204-205 °C. IR (KBr)(ν_{max} cm^{-1}); 3340 (NH), 1220 (P=O), 746 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.90-7.65 (4H, m, Ar-H), 5.80 (1H, s, NH), 3.98-4.10 (8H, m, POC<u>H</u>$_2$CH$_3$), 3.65-3.85 (1H, m, PCH), 1.29 (6H, t, $^3J_{P-H}$ =9.2 Hz, POCH$_2$C<u>H</u>$_3$), 1.02 (6H, t, $^3J_{P-H}$ =8.2 Hz, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 150.2, 149.5, 134.2, 120.4, 118.5, 114.5, 70.1 (d, $^1J_{P-C}$ =158.5 Hz, P-C-H), 60.8 (d, $^2J_{P-C}$ =7.5 Hz, PO<u>C</u>H$_2$CH$_3$), 16.2 (d, $^3J_{P-C}$ =5.6 Hz, POCH$_2$<u>C</u>H$_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 23.2. ESI-MS:(m/z) 424 (100, M^+). Anal.calcd. for $C_{15}H_{26}N_2O_8P_2$: C, 42.46; H, 6.18. Found: C, 41.25; H, 5.99.

Tetraethyl (4-chlorophenylamino)methylene bisphosphonate (***3i***):

White solid, yield 85%, m.p: 130-132 °C. IR (KBr) (ν_{max} cm^{-1}); 3230 (NH), 1250 (P=O), 740 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.54-7.60 (4H, m, Ar-H), 5.20 (1H, s, NH), 3.45-3.75 (1H, m, PCH), 3.87-4.01 (8H, m, POC<u>H</u>$_2$CH$_3$), 1.25-1.20 (12H, m, POCH$_2$C<u>H</u>$_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 145.5, 130.4, 120.4, 115.6, 62.5 (d, $^2J_{P-C}$ =7.5 Hz, PO<u>C</u>H$_2$CH$_3$), 50.2 (d, $^1J_{P-C}$ =162.2 Hz, PCH), 16.4 (d, $^3J_{P-C}$ =5.6 Hz, POCH$_2$<u>C</u>H$_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 24.5. ESI-MS:(m/z) 413 (100, M^+). Anal.calcd. for $C_{15}H_{26}ClNO_6P_2$: C, 43.54; H, 6.33. Found: C, 42.15; H, 6.10.

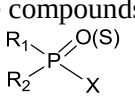
Tetraethyl (4-methylpyridin-2-ylamino)methylene bisphosphonate (***3j***)**:**

White solid, yield 89%, m.p: 145-147 °C. IR (KBr)(ν_{max} cm^{-1}); 3220 (NH), 1210 (P=O), 754 (P-C $_{aliphatic}$). ^{1}H-NMR (400MHz, $CDCl_3$): δ = 6.80-7.82 (3H, m, Ar-H), 5.60 (1H, s, NH), 3.89-3.98 (8H, m, $POC\underline{H}_2CH_3$), 3.45-3.65 (1H, m, PCH), 1.20 (3H, s, Ar-CH_3), 1.28 (6H, t, $^3J_{P-H}$ =9.2 Hz, $POCH_2C\underline{H}_3$), 1.02 (6H, t, $^3J_{P-H}$ =8.2 Hz, $POCH_2C\underline{H}_3$), 1.28-1.22 (12H, m, $POCH_2C\underline{H}_3$). ^{13}C-NMR (100MHz, $CDCl_3$): 156.2, 150.1, 145.2, 120.5, 118.6, 62.5 (d, $^2J_{P-C}$ =7.5 Hz, $PO\underline{C}H_2CH_3$), 68.9 (d, $^1J_{P-C}$ =162.2 Hz, P-C-H), 16.2 (d, $^3J_{P-C}$ =5.6 Hz, $POCH_2\underline{C}H_3$). ^{31}P NMR (161.7MHz, $CDCl_3$): 21.2. ESI-MS:(m/z) 394 (100, M^+). Anal.calcd. for $C_{15}H_{28}N_2O_6P_2$: C, 45.69; H, 7.16. Found: C, 45.10; H, 6.99.

Biological activity:

Antimicrobial activity:

Schrader-Clark [37] proposed that organophosphorous compounds containing the main pharmacophoric structure unit (Figure **1**) may have significant biological activity. Slight variation in pharmacophoric structure (Figure **1**) can have very drastic effects on the bioactive efficiency of organophosphorus compounds (OPC) due to the fact that an OPC substrate is very sensitive to the size, shape and polarity. These chemically and biologically variable parameters which are hard to estimate are involved in deciding "structure-activity" relationship of these compounds.



R1, R2 = Groups which are difficult to displace from phosphorus
X = fairly good leaving group

Figure 1: General Pharmacophoric group of organophosphorous compounds.

Antibacterial activity of ***3a-j*** was tested against the growth of *Staphylococcus aureus* (ATCC 25933), *Bacillus subtilis* (ATCC 55422) (Gram +ve) and *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (31488) (Gram –ve) using the disc diffusion method in nutrient agar medium which are spread by bacteria of 0.1 mL (10^5 CFU/mL) at two different concentrations (100 and 50 µg/mL) in dimethyl formamide (DMF). The plates of 8mm were punched into the agar medium and filled with the title compound solutions to each filter paper disc and DMF was used as control. The plates were incubated at 35 °C and examined for zone of inhibition around each disc after 24 h. The results were compared with the activity of the standard antibiotic penicillin (50 µg/disc). All the compounds showed moderate to high antibacterial activity against both the bacteria (Table **3**).

Table 3: Antibacterial activity of compounds 3a-j (µg/mL).

Compound	Zone of Inhibition (mm)							
	K.Pnenamoniae		*B.subtilis*		*E.coli*		*S.aureus*	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
3a	6	11	7	11	7	9	5	8
3b	9	10	8	12	8	8	6	7
3c	7	12	6	10	7	10	6	8
3d	8	11	7	11	9	11	8	10
3e	6	9	6	10	7	10	7	9
3f	8	10	7	12	6	9	7	8
3g	6	7	7	10	8	10	6	10
3h	9	11	7	12	7	9	5	8
3i	8	12	12	10	6	10	5	7
3j	8	11	8	11	8	9	6	9
Penicillin[a]	12	16	16	15	8	12	8	10

[a]Reference Compound

These compounds were also screened for antifungal activity against *Aspergillus niger* (ATCC 16404) and *Helminthosporium oryzae* (ATCC 11000) species along with the standard antifungal drug *Griseofulvin* utilizing the disc diffusion method at two different concentrations (100 and 50 µg/mL). Fungal cultures

were grown on potato dextrose agar plates (PDA) which were incubated for 72 h at 25 °C and spore suspension was adjusted to 10^5 spores/mL and DMF was used as control without test compounds. Each test was done in triplicate and the mean of the diameter of the inhibition zone was calculated. All the compounds also showed good to moderate antifungal activity against both the fungi (Table **4**).

Table 4: Antifungal activity of compounds 3a-j (µg/mL).

Compound	Zone of Inhibition (mm)			
	Aspergillus niger		*Helminthosporium oryzae*	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
3a	6	9	6	8
3b	8	8	7	7
3c	7	8	6	8
3d	7	10	8	10
3e	6	11	7	8
3f	6	10	9	11
3g	7	11	8	10
3h	8	9	7	9
3i	6	11	8	11
3j	7	10	7	10
Griseofulvin[a]	7	10	9	10

[a]Reference Compound

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