One-Step Synthesis and Bioassay of *N*-Phosphoramidophosphonates

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A simple one-step synthesis was accomplished for the preparation of *N*-phosphoramidophosphonates by a direct reaction of phosphoramidate (1) with heterocyclic aldehydes (2a—j) and dialkyl phosphites at 60—70 °C in the presence of tetramethylguanidine. The tetramethylguanidine not only catalyses this reaction but also helps to form pure products in high yields in lesser time. They exhibited good insecticidal and antioxidant properties.

Key words phosphoramidate; tetramethylguanidine; phosphoramidophosphonate; insecticidal activity; antioxidant activity

The organophosphorus (OP) compounds are one of the largest groups of insecticides in use today and have largely replaced the organochlorine insecticides. The universal use of organophosphorus insecticides has brought about increased benefits in crop production by minimizing pest damage and thereby allowing farmers to adopt better agronomic practices and reap high yields. The OP insecticides have two distinct features. Thy are acutely toxic with a high level of activity inhibiting the vital enzyme cholinesterase, and are chemically unstable and non-persistent in the environment, unlike the chlorinated hydrocarbons.[1,2] Several OP fungicides, such as iprobenfos and edifenphos, are used for the control of fungal disease in plant.[3] They are effective through their activity against chitin synthetase directly through inhibition of the methylation path of phosphatidylcholine synthesis.[4]

In spite of their great practical importance, the detailed mechanism of antioxidant action of organophosphorus compounds and their relationships between chemical structure and antioxidant activity have been comprehensively elucidated only in recent times.[5,6] Depending on their structure, the nature of the polymer to be stabilized and the aging conditions, phosphites and phosphonates may act both as secondary and primary antioxidants.

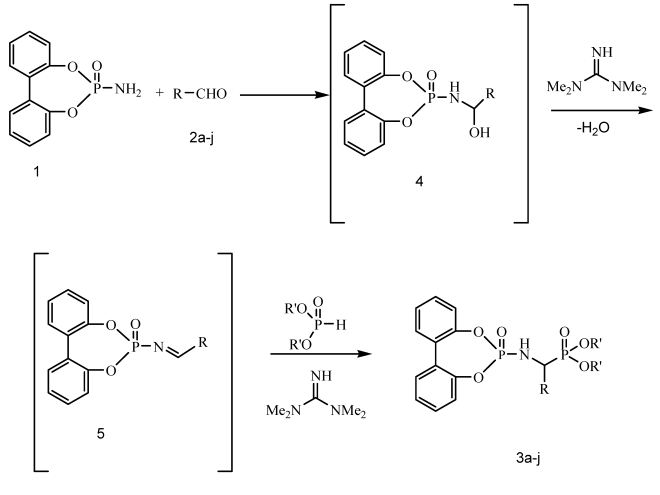
In the present investigation, insecticidal, radical scavenging capacity and antioxidative activity for the newly synthesized compounds are evaluated using two antioxidant methodologies.

Results and Discussion

Reaction of 6-amino-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-oxide **1** with various heterocyclic aldehydes **2a**—**j** and dialkyl phosphites in dry toluene in the presence of tetramethylguanidine (TMG) acts as a catalyst at 60—70 °C for 4 h afforded **3a**—**j**[7] in good yields. In the absence of TMG the reaction takes 10 h and product yields are low. The same reaction in the presence of TMG, is completed in 4 h with high yields 79.2—91.6% (Table 1). The progress of the reaction was monitored by thin layer chromatography (TLC). The chemical structures of **3a**—**j** were confirmed by elemental analyses, IR, ^{1}H-, ^{13}C-, ^{31}P-NMR and mass spectra.

Compounds **3a**—**j** exhibited characteristic IR stretching frequencies in the regions 3410—3380, 1280—1230, 1215—1190 and 740—780 cm^{-1} for N–H, P=O (phosphonate), P=O (phosphepine) and P–C (aliphatic) respectively.[8] The aromatic hydrogens in **3a**—**j** showed a complex multiplet at

Mechanism



Forming the corresponding Schiff's base 5

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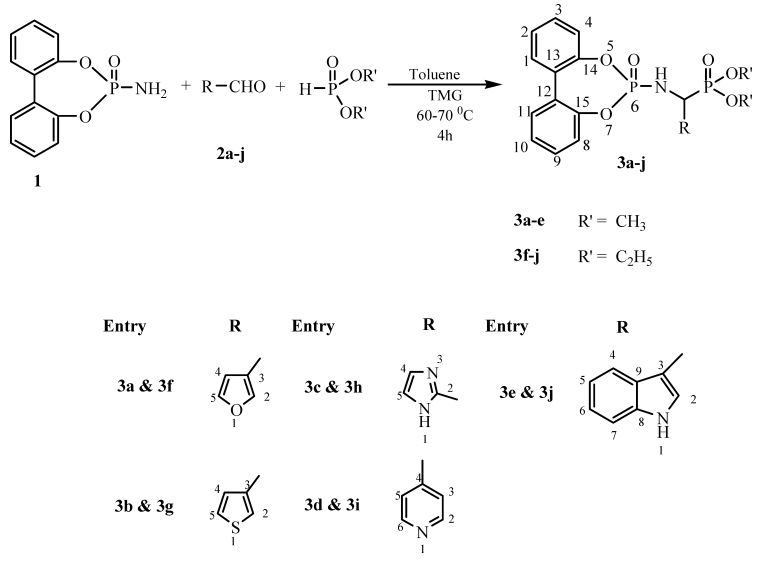
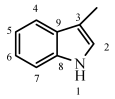
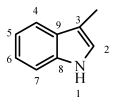


Chart 1

Table 1. TMG Promoted Synthesis of Phosphoramidate Phosphonates **3a**—**j**

Compounds	R	R′	Yield (%)[a]	Yield (%)[b]
3a		CH_3	79.2	62.2
3b		CH_3	83.9	67.5
3c		CH_3	89.6	59.2
3d		CH_3	87.6	61.2
3e		CH_3	91.5	58.9
3f		CH_3CH_2	79.7	62.5
3g		CH_3CH_2	90.3	63.5
3h		CH_3CH_2	91.6	59.8
3i		CH_3CH_2	87.1	61.2
3j		CH_3CH_2	90.9	62.9

a) When the reactions were carried at 60—70 °C in toluene for 1 h in the presence of TMG. *b*) When the reactions were carried at 115—120 °C in toluene without catalyst for 10 h.

δ 6.76—8.21. The P–C–H hydrogen gave a doublet of doublet[9] at δ 5.60—5.80 (dd, ^2J=17.2 Hz, ^2J=11.3 Hz) due to its coupling with phosphorus and the adjacent NH hydrogen. The exocyclic N–H hydrogen gave a singlet at δ 5.80—5.98. The multiplet at δ 3.81—4.21 and the triplet at δ 1.15—1.29 (t, J=7.4 Hz), 1.08—1.16 (t, J=6.9 Hz) were attributed to methylene and methyl hydrogens respectively.[10] The methoxy hydrogens of the dimethyl phosphate moiety resonated as two distinct doublets in the range of δ 3.65—3.89 (d, J=11.2 Hz) 3.35—3.55 (d, J=10.2 Hz) indicating their non-equivalence.[11]

The carbon chemical shifts for P–CH, P–O–CH_3 and P–O–CH_2–CH_3 were observed at δ 45.0, 56.3, 63.4 and 16.4 respectively.[9] The two distinct ^{31}P chemical shifts appeared at δ 40.25 and δ 2.86 are assigned for exocyclic and endocyclo phosphorus.[12] Compounds **3d**, **3e**, **3f**, and **3h** exhibited their respective molecular and characteristic daughter ion peaks in the mass spectra.

The merits of this procedure are: less reaction time, simple work-up and formation of relatively a pure product in high yields. TMG being a strong base (pK_a 10.78) within ability to react with substrates by virtue of its structure, not only catalyses this reaction but also guides its course to avoid formation side products. Therefore, the use of TMG to perform the addition of dialkyl phosphates to unsaturated systems could offer significant advantages especially in terms of experimental simplicity, and easy work-up, high yield, lesser time, handling easy and could represent a convenient tool for the synthesis of a variety of phosphonate synthons.

Bioefficacy Activity Stomach Action: Results presented in Table 2 revealed that among the ten new compounds tested for their toxicity against *Spodotera litura*, **3b**, **3c**, **3g** and **3h** showed high larval mortality of 91.67, 91.67, 83.33 and 83.33% respectively after 72 h of the treatments, with reference to that of chloropyriphos. Compound **3f** recorded 58.33% mortality and other compounds **3a**, **3d**, and **3e** recorded 33.33% mortality. These results further revealed that four compounds (**3b**, **3c**, **3g**, **3h**) possess activity almost on par with chloropyriphos. This is observed that spraying the four compounds (**3b**, **3c**, **3g**, **3h**), on the foliage of crop surface gives complete control of *S. litura* on the third day after spraying. However, *S. litura*, feeding was reduced at the end of second day after spraying. Phytotoxicity symptoms

Table 2. Percent Mortality of *S. litura* Larvae Due to Toxicity of Compounds **3a**—**j** by Stomach Action and Contact Action

Stomach action			Contact action		
Test compounds	Concentration (ppm)	Mean mortality (%)	Test compounds	Concentration (ppm)	Mean mortality (%)
3a	2000	33.33 (35.00)	**3a**	2000	8.33 (10.00)
3b	2000	91.67 (75.00)	**3b**	2000	16.67 (20.00)
3c	2000	83.33 (75.00)	**3c**	2000	16.67 (25.00)
3d	2000	33.33 (35.00)	**3d**	2000	25.00 (20.00)
3e	2000	33.33 (35.00)	**3e**	2000	25.00 (20.00)
3f	2000	58.57 (50.00)	**3f**	2000	16.00 (20.00)
3g	2000	91.67 (80.00)	**3g**	2000	33.33 (35.00)
3h	2000	83.33 (75.00)	**3h**	2000	16.00 (20.00)
3i	2000	25.00 (30.00)	**3i**	2000	20.33 (25.33)
3j	2000	25.00 (30.00)	**3j**	2000	8.33 (10.00)
Chloropyriphos	2000	100.00 (90.00)	Chloropyriphos	2000	91.67 (80.00)
Control (untreated)		0.00 (0.00)	Control (untreated)		0.00 (0.00)
CD at 5%		37.76	CD at 5%		31.88
S.E.M.±		12.87	S.E.M.±		10.87

were not observed with any other test compounds.

Contact action: Results presented in Table 2 revealed that the compound **3g** recorded 33.33% larvae mortality followed by **3d** and **3e** which recorded 25% mortality at 72 h after spraying. Larvae mortality rate was very low in other compounds when compared with that of chloropyriphos with 91.67% mortality. Larvae feeding were normal but slow from third day after spraying.

Antioxidant Testing: The compounds **3a**—**j** were tested for antioxidant property by nitric oxide[13,14] and 1,1-diphenyl-2-picrylhydrazyl (DPPH)[15] methods. The compounds **3b**, **3c**, **3g** and **3h** exhibited high antioxidant property in both nitric oxide and DPPH methods at 100 μM concentrations (Table 3).

Table 3. Nitric Oxide/DPPH Radical Scavenging Activity of **3a**—**j**

% Inhibition at 100 μM		% Inhibition at 100 μM	
Test compounds	Nitric oxide radical	Test compounds	DPPH radical
3a	65.25	**3a**	64.33
3b	85.24	**3b**	89.25
3c	90.11	**3c**	90.28
3d	60.36	**3d**	63.55
3e	65.30	**3e**	68.89
3f	65.23	**3f**	73.25
3g	88.25	**3g**	84.25
3h	89.36	**3h**	87.32
3i	68.36	**3i**	65.26
3j	69.69	**3j**	68.25
BHT	58.62	BHT	67.53

Conclusion

An elegant one-pot synthesis of pohsphoramidophosphonates (**3a**—**j**) by one-step reaction of phospharamidate (**1**), heterocyclic aldehydes (**2a**—**j**) and dialkyl phosphites using TMG as a catalyst is accomplished and good insecticidal and antioxidant properties.

Experimental

General Procedure The melting points were determined in open capillary tubes on a Mel-Temp apparatus and were uncorrected. The IR spectra (ν_{max} in cm^{-1}) were recorded as KBr pellets on Perkin Elmer 1000 unit. The ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on a Varian AMX 400 MHz NMR spectrometers operating at 400 MHz for ^{1}H-, 100.57 MHz for ^{13}C- and 161.7 MHz for ^{31}P. All the compounds were dissolved in $CDCl_3$ or DMSO-d_6 and chemical shifts were referenced to tetramethyl silane (TMS) (^{1}H, ^{13}C) and 85% H_3PO_4 (^{31}P). Micro analyses data were obtained from Central Drug Research Institute, Lucknow, India.

6-Amino-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-oxide (1) The intermediate, 6-chloro-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-oxide[16] (0.05 mol) was made to react with $NaNH_2$ (0.05 mol) in 20 ml of dry toluene at 0 °C over a period of 30 min. Then it was stirred for 5 h at 30 °C 6-amino-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-oxide (**1**) was formed as indicated by TLC. The solvent was removed from the reaction mixture under reduced pressure. The residue was purified by column chromatography on silica gel (80—120 mesh) using petroleum ether–ethylacetate (8 : 2) as eluent. It was recrystallized from 2-propanol to afford pure **1** in 89% yield. mp 166—168 °C.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yldiethyl{3-(furylamino)methyl}phosphonate (3a) Furyl 3-carboxaldehyde (**2a**) (0.005 mol) in anhydrous toluene (20 ml) and stoichiometric amount of TMG was added to the stirred solution of phosphoramide (**1**) (0.005 mol) in anhydrous toluene (15 ml) at room temperature. After stirring for 30 min, dimethyl phosphite (0.005 mol) in anhydrous toluene (20 ml) was added dropwise. Stirring was continued for another 0.5 h at room temperature and then at 60—70 °C for 3 h. The progress of the reaction was monitored by TLC analysis. After completion of the reaction the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (80—120 mesh) using petroleum–ethyl acetate (8 : 3) as eluent. It was recrystallized from 2-propanol to afford pure **3a** in 79.2% yield. mp 189—191 °C. Other compounds (**3b**—**j**) were prepared by using this above procedure.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepine-6-yl-dimethyl-3-furyl Aminomethylphosphonate (**3a**): Colorless solid, mp 210—212 °C. IR (KBr) cm^{-1}: 3420 (NH), 1270 (P=O, phosphonate), 1190 (P=O, phosphepine), 752 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.80—7.82 (10H, m, Ar–H), 5.72 (d, J=8.9 Hz, Ar–H), 5.92 (1H, s, N–H), 5.72 (1H, dd, $^2J_{P-H}$=14.3 Hz, $^3J_{P-H}$=11.4 Hz), 3.68 (3H, d, $^3J_{P-H}$=10.2 Hz, P–OCH_3), 3.45 (3H, d, $^3J_{P-H}$=9.2 Hz, P–OCH_3). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 28.2 (P=O, phosphonate), 7.2 (P=O, phosphepine). *Anal.* Calcd for $C_{19}H_{19}NO_7P_2S$: C, 52.42; H, 4.40; N, 3.22. Found: C, 52.38; H, 4.36; N, 3.18%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-dimethyl-3-thienyl Aminomethylphosphonate (**3b**)**:** White solid, mp 180—182 °C; IR (KBr) cm^{-1}: 3410 (NH), 1265 (P=O, phosphonate), 1210 (P=O, phosphepine); 756 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.71—7.83 (11H, m, Ar–H), 5.80 (1H, s, N–H), 5.60 (1H, dd, ^2J=12.9 Hz, $^3J_{P-H}$=10.0 Hz), 3.65 (3H, d, $^3J_{P-H}$=11.4 Hz), P–OCH_3), 3.55 (3H, d, $^3J_{P-H}$=11.3 Hz, P–OCH_3). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 45.3 (P=O, phosphonate), 7.8 (P=O, phosphepine). *Anal.* Calcd. for $C_{19}H_{19}NO_6P_2S$: C, 50.66; H, 4.24; N, 3.10. Found: C, 50.58; H, 4.18; N, 3.06 %.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-dimethyl-1*H*-2-imidazol Aminomethylphosphonate (**3c**): White solid, mp 198—200 °C; IR (KBr) cm^{-1}: 3390 (NH), 1260 (P=O, phosphonate), 1198 (P=O, phosphepine); 745 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.89 (1H, s, imidazole N–H), 6.85—7.80 (10H, m, Ar–H), 5.90 (1H, s, N–H), 5.60 (1H, dd, ^2J=14.2, $^3J_{P-H}$ 11.8 Hz, P–CH), 3.71 (3H, d, $^3J_{P-H}$=10.3 Hz, P–OCH_3), 3.62 (3H, d, $^3J_{P-H}$=9.2 Hz, P–OCH_3). ^{31}P-NMR (161.1 MHz, DMSO-d_6) δ: 21.2 (P=O, Phosphonates), 3.2 (P=O, phosphepine). *Anal.* Calcd for $C_{18}H_{19}N_3O_6P_2$: C, 49.67; H, 4.40; N, 9.65. Found: C, 49.62; H, 4.35; N, 9.60%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-dimethyl-4-pyridyl Aminomethylphosphonate (**3d**): Colorless solid, mp 190—192 °C. IR (KBr) cm^{-1}: 3419 (NH), 1226 (P=O, phosphonate), 1150 (P=O, phosphepine); 751 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.82—7.82 (12H, m, Ar–H), 5.92 (1H, s, N–H), 5.62 (1H, dd, $^2J_{P-H}$=12.2 Hz, $^3J_{P-H}$=10.3 Hz, P–CH), 3.65 (3H, d, $^3J_{P-H}$=12.8 Hz), 3.55 (3H, d, $^3J_{P-H}$=10.2 Hz, P–OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 129.2 (C-1, C-11), 122.6 (C-2, C-10), 127.5 (C-3, C-9), 118.3 (C-4, C-8), 126.1 (C-12, C-13), 149.9 (C-14, C-15), 149.5 (C′-2, C′-6), 125.4 (C′-3, C′-5), 138.8 (C′-4), 51.5 (d, ^2J=7.3 Hz, P–O–CH_3), 45.3 (P–CH). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 28.6 (P=O, phosphonate), 2.6 (P=O, phosphepine). LC-MS; *m/z* (%) 447 (60) (M+1). *Elemental Anal.* Calcd for $C_{20}H_{20}N_2O_6P_2$: C, 53.82; H, 4.52; 6.28. Found: C, 53.77; H, 4.48; N, 6.24%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepione-6-yl-dimethyl-3-indole Aminomethylphosphonate (**3e**): Pale yellow crystals, mp 190—192 °C. IR (KBr) cm^{-1}: 3435 (NH), 1267 (P=O, phosphonate), 1228 (P=O, phosphepine), 764 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.86 (1H, s Indole N–H) 6.81—8.15 (13H, m, Ar–H), 5.95 (1H, s, N–H), 5.69 (1H, dd, ^2J=15.2, $^3J_{P-H}$=11.6 Hz, P–CH), 3.65 (3H, d, $^3J_{P-CH}$=12.2 Hz, P–OCH_3), 3.40 (3H, d, $^3J_{P-CH}$=12.2 Hz, P–OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 130.3 (C-1, C-11), 122.4 (C-2, C-10), 129.9 (C-3, C-9), 119.9 (C-4, C-8), 126.4 (C-12, C-13), 150.3 (C-14, C-15), 121.7 (C′-2), 121.4 (C′-3) 123.6 (C′-4), 124.7 (C′-5), 123.6 (C′-6), 128.7 (C′-7), 139.9 (C′-8), 129.2 (C′-9), 53.2 (d, $^2J_{P-C}$ 6.5, P–OCH_3), 40.1 (P–CH). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 45.6 (P=0, phosphonate), 1.42 (P=O, phosphepine). LC-MS; *m/z* (%), 486 (30) (M+2). *Anal.* Calcd for $C_{23}H_{22}N_2O_6P_2$: C, 57.03; H, 4.58; N, 5.78. Found: C, 57.00; H, 4.54; N, 5.74%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-diethyl-3-furyl Aminomethylphosphonate (**3f**): Colorless solid, mp 189—191 °C. IR (KBr) cm^{-1}: 3410 (NH), 1259 (P=O, phosphonate), 1210 (P=O, phosphepine), 760 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.81—7.80 (10H, m, Ar–H), 5.98 (1H, s, NH), 5.80 (1H, d, *J*=9.8 Hz, Ar–H), 5.60 (1H, dd, $^2J_{P-H}$=17.1 Hz, $^3J_{P-H}$=11.2 Hz, P–CH), 3.82—4.21 (4H, m, P–O<u>CH</u>$_2$–CH_3), 1.15 (3H, t, ^3J=7.3 Hz, P–OCH_2–<u>CH</u>$_3$), 1.10 (3H, t, ^3J=6.5 Hz, P–OCH_2–C<u>H</u>$_3$). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 128.1 (C-1, C-11), 120.1 (C-2, C-10), 128.1 (C-3, C-9), 118.1 (C-4, C-8), 128.2 (C-12, C-13), 151.2 (C-14, C-15), 139.4 (C′-2), 120.8 (C′-3), 109.2 (C′-4), 146.8 (C′-5), 64.5 (d, $^2J_{P-C}$=7.6 Hz, P–O<u>C</u>H_2CH_3), 46.1 (P–C) 17.5 (d, $^3J_{P-C}$=5.8 Hz, P–OCH_2–<u>C</u>H_3). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 28.2 (P=O, phosphonate), 3.80 (P=O, phosphepine). LC-MS; *m/z* (%): 486 (100) (M+Na). *Anal.* Calcd for $C_{21}H_{23}NO_7P_2$: C, 54.47; H, 5.00; N, 3.02 Found: C, 54.34; H, 4.96, N, 2.98%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-diethyl-3-thineyl Aminomethylphosphonate (**3g**): Colorless solid, mp 191—193 °C. IR (KBr) cm^{-1}: 3410 (NH), 1260 (P=O, phosphonate), 1190 (P=O, phosphepine), 755 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.82—7.70 (11H, m, Ar–H), 5.93 (1H, s, NH), 5.60 (1H, d, $^2J_{P-H}$=16.8 Hz, $^3J_{P-H}$=11.2 Hz, P–CH), 3.81—4.10 (4H, m, P–O<u>CH</u>$_2$–CH_3), 1.18 (3H, t, ^3J=7.8 Hz, P–OCH_2–C<u>H</u>$_3$), 1.10 (3H, t, ^3J=6.6 Hz, P–OCH_2–C<u>H</u>$_3$). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 29.2 (P=O, phosphonate), 2.90 (P=O, phosphepine). *Anal.* Calcd. for $C_{21}H_{23}NO_6P_2S$: C, 52.61; H, 4.84; N, 2.92. Found: C, 52.58; H, 4.79, N, 2.88%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-diethyl-1*H*-2-imidazolyl Aminomethylphosphonate (**3h**): Colorless solid, mp 191—193 °C. IR (KBr) cm^{-1}: 3380 (NH), 1250 (P=O, phosphonate), 1195 (P=O phosphepine), 750 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.81—7.80 (10H, m, Ar–H), 5.90 (1H, s, NH), 5.60 (1H, dd, $^2J_{P-H}$=17.5 Hz, $^3J_{P-H}$=10.8 Hz, P–CH), 3.79—4.10 (4H, m, P–O<u>CH</u>$_2$$CH_3$), 1.18 (3H, t, ^3J=7.4 Hz, P–OCH_2–C<u>H</u>$_3$), 1.08 (3H, t, ^3J=6.6 Hz, P–OCH_2–C<u>H</u>$_3$). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 129.2 (C-1, C-11), 120.8 (C-2, C-10), 129.8 (C-3, C-9), 117.8 (C-4, C-8), 127.8 (C-12, C-13), 149.8 (C-14, C-15), 148.9 (C′-2), 125.3 (C′-4) 126.5 (C′-5), 63.5 (d, $^2J_{POC}$=7.3 Hz, P–O<u>C</u>H_2–CH_3), 45.1 (P–C); 15.3 (d, $^3J_{POCC}$=6.1 Hz, P–OCH_2–<u>C</u>H_3). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 28.1 (P=O, phosphonate), 6.8 (P=O, phosphepine). LC-MS MS; *m/z* (%) 463 (55) (M+H). *Anal.* Calcd for $C_{20}H_{23}N_3O_6P_2$: C, 51.84; H, 5.00; N, 9.07. Found: C, 51.79; H, 4.95, N, 9.02%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-diethyl-4-pyridyl Aminomethylphosphonate (**3i**): Colorless solid, mp 188—190 °C. IR (KBr) cm^{-1}: 3420 (NH), 1240 (P=O, phosphonate), 1208 (P=O, phosphepine), 759 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.82—8.12 (12H, m, Ar–H), 5.92 (1H, s, NH), 5.63 (1H, dd, $^2J_{P-H}$=17.2 Hz, $^3J_{P-H}$=11.3 Hz, P–CH), 3.72—3.98 (4H, m, P–O<u>CH</u>$_2$–CH_3), 1.16 (3H, t, ^3J=7.5 Hz, P–OCH_2–C<u>H</u>$_3$), 1.08 (3H, t, ^3J=6.4 Hz, P–OCH_2–C<u>H</u>$_3$). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 35.2 (P=O, phosphonate), 4.8 (P=O, phosphepine). *Anal.* Calcd. for $C_{22}H_{24}N_2O_6P_2$: C, 55.70; H, 5.10; N, 5.91. Found: C, 55.65; H, 5.05; N, 5.87%.

6-Oxo-6λ^5-dibenzo[*d*,*f*][1,3,2]dioxaphosphepin-6-yl-diethyl-1*H*-3-indolyl Aminomethylphosphonate (**3j**): Colorless solid, mp 198—200 °C. IR (KBr) cm^{-1}: 3410 (NH), 1280 (P=O, phosphonate), 1211 (P=O, phosphepine); 746 (P–$C_{aliphatic}$). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.80—8.10 (13H, m, Ar–H), 5.91 (1H, s, NH), 5.61 (1H, dd, $^2J_{P-H}$=17.2 Hz, $^3J_{P-H}$=11.2 Hz, P–CH), 3.82—3.98 (4H, m, P–O<u>CH</u>$_2$–CH_3), 1.15 (3H, t, ^3J=7.4 Hz, P–OCH_2–C<u>H</u>$_3$), 1.10 (3H, t, ^3J=6.6 Hz, P–OCH_2–C<u>H</u>$_3$). ^{31}P-NMR (161.7 MHz, DMSO-d_6) δ: 33.2 (P=O, phsophonate), 4.8 (P=O, phsophepine). *Anal.* Calcd for $C_{25}H_{26}N_2O_6P_2$: C, 58.60; H, 5.11; N, 5.47. Found: C, 58.56; H, 5.08, N, 5.42%.

Bioassay. Insecticidal Activity Compounds **3a**—**j** were tested for their bioefficacy[17,18] on tobacco caterpillar, *Spodoptera litura*. *S. litura* is a major pest on many crops like groundnut, cowpea, greengram, castor, and cotton, *etc*. Each compound was dissolved in mercapto ethanol for preparing the stock solutions and the compounds were tested at 2000 ppm each. Chloropyriphos an organophosphorus insecticide is used as reference compound for the control of *S. litura*.

There were twelve treatments including a control which was maintained without insecticide application. Each treatment consisted of eight third instar larvae with three replications. Just before the insecticidal treatment the larvae were transferred carefully with a hair brush into a clean and dry Petri dish of 100×15 mm at the rate of eight larvae per Petri dish covered with lids.

Two methods of testing were done to know the contact and stomach action of the compounds on *S. litura*. In one method castor leaves were cut into a disc size of 100×15 mm so as to spread in the Petri dish. The compounds **3a**—**j** and chloropyriphos were sprayed on to the leaf disc before feeding it to the larvae in the Petri dish for the stomach action. In another method the compounds were sprayed directly on to the body of larvae kept in the Petri dish and later on transferred to a clean Petri dish with fresh castor leaf disc for determining the contact action. Potters' spraying tower was used to spray the insecticide in both the methods. Larvae mortality counts were taken at regular interval of 24 h after imposing the treatments. The larvae which were moribund and did not show any movement were considered as dead.

Antioxidant Testing The compounds **3a**—**j** are tested for antioxidant property by nitric oxide and DPPH methods.

Assay for Nitric Oxide (NO) Scavenging Activity Sodium nitroprusside (5 μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentration of test compounds dissolved in a suitable solvent (dioxane/methanol) and tubes were incubated at 25 °C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5 ml of incubation solution was taken and diluted with 0.5 ml of griess reagent (1% sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *O*-phosphoric acid dissolved in distilled water). The absorbance of the chordophone formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at 546 nm. The experiment was repeated in triplicate.

Reduction of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Free Radical (DPPH Method) The nitrogen centered stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at λ 517 nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric, with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties.

The solutions of test compounds (100 μM) were added to DPPH (100 μM) in dioxane/ethanol. The tubes were kept at an ambient temperature for 20 min and the absorbance was measured at λ 517 nm. The difference between the test and the control experiments was taken and expressed as the per cent scavenging of the DPPH radical.

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