Synthesis, Anti-bacterial and Anti-oxidant Properties of Thiadiazaphosphol-2-ones

Avula Balakrishna,[a] Mudamala Veera Narayana Reddy,[a] Sandip Kumar Nayak,[b] Manubolu Manjunath,[a] Chichili Devendranath Reddy,[a] and Cirandur Suresh Reddy*,[a]

[a] *Department of Chemistry, Biochemistry, S.V. University; Tirupati 517 502, India: and* [b] *Bio-Organic Division, BARC; Mumbai 400 085, India.* Received May 31, 2008; accepted July 26, 2008; published online July 30, 2008

4-Amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (1) underwent facile condensation with various phosphorus dichlorides (2a—j) in the presence of triethylamine in dry tetrahydrofuran at 60—65 °C and afforded corresponding thiadiazaphosphol-2-ones (3a—j). Their chemical structures were characterized using IR, ^{1}H-, ^{13}C-, ^{31}P-NMR and Mass spectral studies. All the title compounds were screened for antioxidant properties by radical scavenging methods such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), hydroxyl and lipid peroxidation. They exhibited potent *in vitro* antioxidant activity dose dependently. Their bioassay showed them to possess significant antibacterial activity.

Key words thiadiazaphosphol-2-one; spectral analysis; *in vitro* antioxidant activity; antibacterial activity

Syntheses of 1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]-thiadiaza-phosphol-2-one derivatives are presently reported. 1,2,4 Triazole derivatives are reported to possess a broad spectrum of biological activities such as systemic pesticides, antifungal/microbial/lukemic/parasitic/viral/inflammatory/tumor/hypertensive/oxidant and anti-human immunodeficiency virus properties.[1—8] They also have hypoglycemic, hypocholesteremic activity. This background prompted synthesis of new thiadiazaphosphol-2-ones as continuation of our work in phosphorus heterocycles.[9]

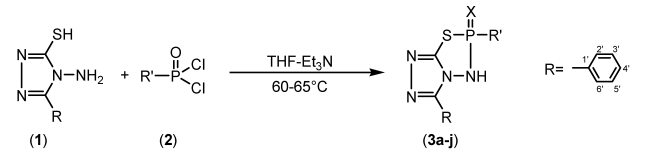
Antioxidants are widely studied for their capacity to protect organisms and cells from damage induced by oxidative stress during metabolism. Search for active components that prevent or reduce the impact of oxidative stress on cells[10] is a contemporary field. Exogenous chemicals in food system and endogenous metabolic processes in human body produce highly reactive free radicals, especially oxygen derived ones. They are capable of oxidizing biomolecules and cause cell death and consequently cause tissue damage. Free radical oxidative processess also play a significant pathological role in causing human diseases. Many disease manifestations such as cancer, emphysema, cirrhosis, atherosclerosis and arthritis have been correlated with oxidative tissue damage. Also, excessive generation of reactive oxygen species (ROS) induced by various stimuli leads to variety of pathophysiological abnormalities such as inflammation, diabetes, genotoxicity and cancer.[11] In the present investigation, radical scavenging and antioxidative activity for the newly synthesized compounds are evaluated using three antioxidant methodologies.

Results and Discussion

Compounds **3a**—**j** were synthesized by condensation of phosphonic dichlorides **2a**—**d** substituted phosphorodichloridates **2e**—**i** and bis(2-chloroethyl)amine dichloride **2j** with 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol **1** in the presence of a base in dry tetrahydrofuran (THF)[12] (Chart 1).

Characteristic IR absorptions were observed in the regions 1219—1254 cm^{-1} for P=O and 3170—3260 cm^{-1} for P–N–H stretching frequencies for **3a**—**j**.[13] The aromatic protons resonated as multiplets at δ 6.91—8.11 in their ^{1}H-NMR spectra. The P–N–H proton chemical shift appeared as a singlet at δ 5.44—5.82.[14] The ^{13}C-NMR chemical shifts for them appeared in the expected region.[13] ^{31}P-chemical shifts occurred at δ 16.34—+14.31.[15]

The radical scavenging capacity of **3a**—**j** was evaluated by methods such as 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Fig. 1), lipid peroxidation (Fig. 2) and hydroxyl radical scavenging techniques (Fig. 3). **3g**, **3h** and **3i** displayed appreciable antioxidant activity. This points to the fact that any electron withdrawing substituent moiety at the phosphorus in **3a**—**j** appears to prevent to some extent oxidative metabolic



(1) (2) (3a-j)

Entry	R′	X	Entry	R′	X
2a & 3a	-$CH_2CH_2CH_3$	O	2f & 3f	-OC_2H_5	O
2b & 3b		O	2g & 3g		O
2c & 3c		S	2h & 3h		O
2d & 3d		Se	2i & 3i		O
2e & 3e	-OCH_3	O	2j & 3j	-N-$(CH_2CH_2Cl)_2$	O

Chart 1

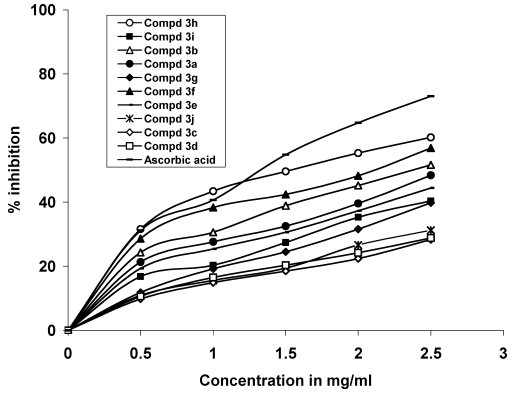


Fig. 1. DPPH Radical Scavenging Activity of Compounds

* To whom correspondence should be addressed. e-mail: csrsvu@gmail.com

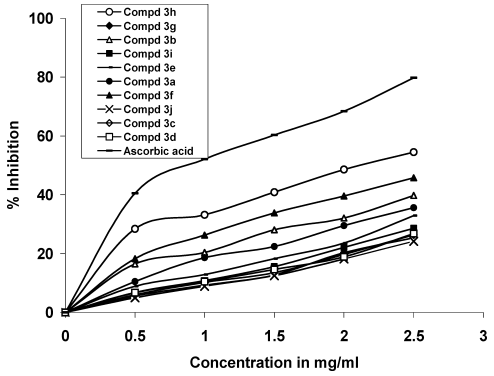


Fig. 2. Effect of Compounds on Ferric Ion and Ascorbic Acid Induced Lipid Peroxidation

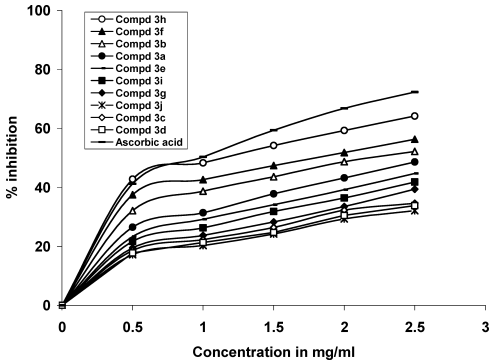


Fig. 3. Hydroxyl Radical Scavenging Activity

pathways in the living systems. In conclusion, synthesis of a series novel thiadiazaphosphol-2-ones in high yields was accomplished. Some of them were found to possess good antioxidant and significant antimicrobial activity. These results encourage further *in vivo* studies and explore their possible therapeutic applications.

Experimental

General The melting points were determined in open capillary tubes on a Mel-Temp apparatus and were uncorrected. Elemental analyses were performed by Central Drug Research Institute, Lucknow, India. The IR spectra were recorded as KBr pellets on PERKIN-ELMER 1000 unit. All ^{1}H- and ^{13}C-NMR spectra were recorded on a VARIAN XL-300 spectrometer operating at 300 MHz for ^{1}H and 75.46 MHz for ^{13}C. ^{31}P-NMR spectra were recorded on a VARIAN XL-spectrometer operating at 161.89 MHz. The compounds were dissolved in DMSO-d_6 and chemical shifts were referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P). Mass spectral data was recorded on FAB-MS instrument at 70 eV with a direct inlet system.

4-Amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**1**) and various phosphorodichloridates/phosphorus dichlorides **2a**—**j** were procured from Sigma-Aldrich Chemical Company, Milwaukee, U.S.A. and were used without further purification.

General Procedure for Preparation of 2-(Substituted)-6-phenyl-1,2-dihydro-2λ^5-[1,2,4]-triazolo-[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (3a—j) A solution of respective phosphorodichloridate **2a**—**j** (0.003 mol, 0.48 ml) in dry THF was added drop wise over a period of 20 min to a stirred solution of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol **1** (0.003 mol, 570 mg) in the presence of triethylamine (0.003 mol, 0.42 ml) in 60 ml of dry THF. After completion of addition, the temperature was increased to 60—65 °C and stirring was continued for an additional 4—6 h. Progress of the reaction was monitored by thin layer chromatography analysis using silica gel as adsorbent and ethyl acetate–hexane (1 : 2) mixture as eluent. Product was isolated from the reaction mixture by separating triethylamine hydrochloride by filtration and evaporation of the filtrate under reduced pressure. The residue was purified by washing with water followed by recrystallization from 2-propanol.

6-Phenyl-2-propyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3a**): Yield was found to be 72%, mp 128—130 °C. IR (KBr) cm^{-1}: 1247, 3173. ^{1}H-NMR (DMSO-d_6): δ 4.2 (2H, m), 2.3 (2H, m), 1.3 (3H, t), 7.97—8.00 (t, 2H), 7.57—7.59 (t, 3H), 5.61 (1H, s). ^{31}P-NMR data: δ −13.41. *Anal.* Calcd for $C_{11}H_{13}N_4OPS$: C, 47.14; H, 4.68; N, 19.99. Found C, 47.05; H, 4.61; N, 19.92.

2,6-Diphenyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3b**): Yield was found to be 75%, mp 166—168 °C. IR (KBr) cm^{-1}: 1236, 3200. ^{1}H-NMR (DMSO-d_6): δ 7.28—8.77 (10H, m), 5.78 (1H, s). ^{13}C-NMR data: 131.01 (C-1′), 130.47 (C-2′), 129.82 (C-3′), 128.47 (C-4′), 129.82 (C-5′), 130.47 (C-6′), 131.01 (C-1″), 128.0 (C-2″), 127.68 (C-3″), 126.95 (C-4″), 127.68 (C-5″), 128.0 (C-6″), 161.12 (C-3), 148.24 (C-5). ^{31}P-NMR data: δ 13.76. *Anal.* Calcd for $C_{14}H_{11}N_4OPS$: C, 53.50; H, 3.53; N, 17.83. Found C, 53.40; H, 3.52; N, 17.72.

2,6-Diphenyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-thione (**3c**): Yield was found to be 63%, mp semi-solid. IR (KBr) cm^{-1}: 806 (P=S), 3179. ^{1}H-NMR (DMSO-d_6): δ 7.42—8.03 (10H, m), 5.73 (1H , s). ^{31}P-NMR data: δ −15.34. *Anal.* Calcd for $C_{14}H_{11}N_4PS_2$: C, 50.90; H, 3.36; N, 16.96. Found C, 50.81; H, 3.29; N, 16.89.

2,6-Diphenyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-selone (**3d**): Yield was found to be 65%, mp semi-solid. IR (KBr) cm^{-1}: 685 (P=Se); 3209. ^{1}H-NMR (DMSO-d_6): δ 7.51—8.01 (10H, m), 5.82 (1H, s). ^{13}C-NMR data: 131.11 (C-1′), 130.58 (C-2′), 129.64 (C-3′), 128.12 (C-4′), 129.72 (C-5′), 130.34 (C-6′), 130.87 (C-1″), 129.01 (C-2″), 126.92 (C-3″), 126.01 (C-4″), 126.92 (C-5″), 129.01 (C-6″), 161.02 (C-3), 148.72 (C-5). ^{31}P-NMR data: δ −14.41. *Anal.* Calcd for $C_{14}H_{11}N_4PSSe$: C, 44.57; H, 2.94; N, 14.84. Found C, 44.46; H, 2.89; N, 14.78.

2-Methoxy-6-phenyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3e**): Yield was found to be 68%, mp semi-solid. IR (KBr) cm^{-1}: 1254, 3170. ^{1}H-NMR (DMSO-d_6): δ 7.95—8.03 (t, 2H), 7.54—7.56 (3H, t), 3.12 (3H, s), 5.52 (1H, s). ^{13}C-NMR data: 149.21 (C-1′), 134.12 (C-2′), 128.12 (C-3′), 130.34 (C-4′), 127.21 (C-5′), 134.12 (C-6′), 61.24 (O–$\underline{C}H_3$), 160.12 (C-3), 149.21 (C-5). ^{31}P-NMR data: δ −13.27. *Anal.* Calcd for $C_9H_9N_4O_2PS$: C, 40.30; H, 3.38; N, 20.89. Found C, 40.21; H, 3.32; N, 20.81.

2-Ethoxy-6-phenyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3f**): Yield was found to be 80%, mp 148—150 °C. IR (KBr) cm^{-1}: 1219, 3210. ^{1}H-NMR (DMSO-d_6): δ 7.98—8.00 (t, 2H), 7.51—7.52 (3H, t), 3.84 (q, 2H), 3.06—3.07 (t, 3H), 5.76 (1H, s). ^{31}P-NMR data: δ 14.31. FAB-MS *m*/*z*: 283 (M+1), *Anal.* Calcd for $C_{10}H_{11}N_4O_2PS$: C, 42.55; H, 3.93; N, 19.85. Found C, 42.46; H, 3.93; N, 19.77.

2-(2-Chlorophenoxy)-6-phenyl-1,2-dihydro-2λ^5-[1,2,4]-triazolo-[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3g**): Yield was found to be 72%, mp 240—242 °C. IR (KBr) cm^{-1}: 1234, 3205. ^{1}H-NMR (DMSO-d_6): δ 6.91—8.02 (9H, m), 5.74 (1H, s). ^{13}C-NMR data: 149.09 (C-1′), 128.48 (C-2′), 128.01 (C-3′), 130.43 (C-4′), 128.01 (C-5′), 128.48 (C-6′), 149.41 (C-1″), 125.76 (C-2″), 123.89 (C-3″), 121.2 (C-4″), 123.35 (C-5″), 122.50 (C-6″), 166.81 (C-3), 128.48 (C-5). ^{31}P-NMR data: δ −16.43. FAB-MS *m*/*z*: 365 (M+1). *Anal.* Calcd for $C_{14}H_{10}ClN_4O_2PS$: C, 46.10; H, 2.76; N,15.36. Found C, 46.01; H, 2.69; N, 15.31.

2-(4-Chlorophenoxy)-6-phenyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3h**): Yield was found to be 73%, mp semi-solid. IR (KBr) cm^{-1}: 1229, 3217. ^{1}H-NMR (DMSO-d_6): δ 6.94—8.01 (9H, m), 5.72 (1H, s). ^{13}C-NMR data: 147.91 (C-1′), 128.44 (C-2′), 126.41 (C-3′), 129.32 (C-4′), 126.13 (C-5′), 128.44 (C-6′), 148.18 (C-1″), 120.12 (C-2″), 119.18 (C-3″), 115.12 (C-4″), 119.18 (C-5″), 120.12 (C-6″), 165.71 (C-3), 127.45 (C-5). ^{31}P-NMR data: δ 16.39. *Anal.* Calcd for $C_{14}H_{10}ClN_4O_2PS$: C, 46.10; H, 2.76; N, 15.36. Found C, 46.02; H, 2.71; N, 15.30.

6-Phenyl-2-(4-nitrophenoxy)-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3i**): Yield was found to be 70%, mp 164—166 °C. IR (KBr) cm^{-1}: 1248, 3260. ^{1}H-NMR (DMSO-d_6): δ 7.192—8.11 (m, 9H), 5.70 (1H, s). ^{31}P-NMR data: δ 13.82. *Anal.* Calcd for $C_{14}H_{10}N_5O_4PS$: C, 44.81; H, 2.69; N, 18.61. Found C, 44.72; H, 2.61; N, 18.53.

2-[Bis(2-chloroethyl)amino]-6-phenyl-1,2-dihydro-2λ^5-[1,2,4]triazolo[4,3-*d*][1,3,4,2]thiadiazaphosphol-2-one (**3j**): Yield was found to be 70%, mp semi-solid. IR (KBr) cm^{-1}: 1252, 3181. ^{1}H-NMR (DMSO-d_6): δ 7.97—8.01 (t, 2H), 7.53—7.55 (t, 3H), 5.44 (1H, s). ^{31}P-NMR data: δ −12.28. *Anal.* Calcd for $C_{12}H_{14}Cl_2N_5OPS$: C, 38.11; H, 3.73; N, 18.52. Found C, 38.02; H, 3.73; N, 18.47.

Antioxidant Activity. 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical Scavenging Activity The free radical scavenging activity of **3a**—**j** and

Table 1. Antibacterial Activity (Diameter of Zone of Inhibition in mm) of Compounds **3a**—**j** (μg/ml)

Bacteria	**3a**		**3b**		**3c**		**3d**		**3e**		**3f**		**3g**		**3h**		**3i**		**3j**		Chloramphenicol	
	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100
E. coli	16	23	10	13	8	11	7	10	8	11	13	17	7	10	6	8	7	10	12	16	—	22
S. typhimurium	15	18	8	12	7	10	8	11	9	12	12	15	8	11	8	11	6	8	13	15	—	22
S. aureus	15	20	10	12	8	11	5	8	7	10	14	16	8	12	7	10	6	8	14	17	—	25
B. faecalis	17	22	10	13	7	10	5	8	8	10	13	17	8	12	8	10	6	8	13	16	—	26

a) Each well contains 50 and 100 μg of compound.

Table 2. MIC of Compounds **3a**—**j** (μg/ml)

Bacteria	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**
E. coli	80	350	410	600	380	140	560	640	570	120
S.typhimurium	90	290	460	570	410	130	440	520	540	130
S. aureus	70	410	360	510	330	170	520	610	490	110
B. faecalis	70	370	440	530	460	120	400	560	470	130

ascorbic acid were measured by the method of Blois (1958),[16] and the data are presented in Fig. 1. The antioxidant activity of these compounds was expressed as IC_{50} (inhibitory concentration, 50%). DPPH forms a stable molecule on accepting an electron or a hydrogen and thus found application in the determination of radical scavenging and antioxidant activity.[17,18] In the case of triazole thiadiazaphosphol-2-ones derivatives **3h** showed highest DPPH scavenging activity with IC_{50} of 1.51 mg/ml when compared with other compounds. The remaining compounds exhibited DPPH radical scavenging activity in the following order: **3i** (IC_{50} 2.07 mg/ml)>**3b** (IC_{50} 2.42 mg/ml)>**3a** (IC_{50} 2.58 mg/ml)>**3g** (IC_{50} 2.81 mg/ml)>**3f** (IC_{50} 3.09 mg/ml)>**3e** (IC_{50} 3.14 mg/ml)>**3j** (IC_{50} 4.0 mg/ml)>**3c** (IC_{50} 4.41 mg/ml)>**3d** (IC_{50} 4.34 mg/ml) and was significant ($p<0.001$) when compared with that of ascorbic acid (IC_{50} 1.36 mg/ml).

$$\text{DPPH scavenged (\%)} = \frac{(A_{\text{cont}} - A_{\text{test}})}{A_{\text{cont}}} \times 100$$

where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample.

Lipid Peroxidation Assay Lipid peroxidation was induced by Fe^{2+}–ascorbate complex system in rat red blood cells and estimated as thiobarbituric acid reacting substances (TBARS) by the method of Buege and Aust (1978).[19] Experiments *in vitro* lipid peroxidation were carried out to clarify the mode of the protective effect of the triazole compounds against oxidative stress-induced cell damage. The inhibition of lipid peroxidation has been used as a model to elucidate antioxidant activity. According to the results obtained, **3h** significantly ($p<0.001$) inhibited the ferric ion plus ascorbic acid in rat red blood cells (Fig. 2). The remaining compounds exhibited hydroxyl radical scavenging activity in the following order: **3g** (IC_{50} 2.72 mg/ml)>**3b** (IC_{50} 3.14 mg/ml)>**3i** (IC_{50} 3.51 mg/ml)>**3e** (IC_{50} 3.79 mg/ml)>**3a** (IC_{50} 4.37 mg/ml)>**3f** (IC_{50} 4.92 mg/ml)>**3j** (IC_{50} 5.16 mg/ml)>**3c** (IC_{50} 4.73 mg/ml)>**3d** (IC_{50} 4.66 mg/ml) and the results are significant ($p<0.001$) when compared with that of ascorbic acid (IC_{50} 0.98 mg/ml).

Hydroxyl Radical Scavenging Activity It was carried out by measuring the competition between deoxyribose and the compounds that generate hydroxyl radicals from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. Attack of the hydroxyl radicals on deoxyribose led to thiobarbituric acid-reactive substances (TBARS) formation. The formed TBARS were measured by the method of Ohkawa *et al.* (1979).[20] The hydroxyl radical is the most reactive oxygen species (ROS) that attacks almost every molecule in the body. It initiates the peroxidation of cell membrane lipids[21,22] yielding malondialdehyde, which is mutagenic and carcinogenic.[23] Even though the triazoles are known to scavenge the hydroxyl radical, the compound **3h** showed significant hydroxyl radical scavenging activity with IC_{50} of 1.03 mg/ml when compared with other compounds. The remaining compounds exhibited hydroxyl radical scavenging activity in the following order respectively: **3f** (IC_{50} 1.93 mg/ml)>**3b** (IC_{50} 2.05 mg/ml)>**3a** (IC_{50} 2.57 mg/ml)>**3e** (IC_{50} 2.79 mg/ml)>**3i** (IC_{50} 2.99 mg/ml)>**3g** (IC_{50} 3.17 mg/ml)>**3j** (IC_{50} 3.89 mg/ml)>**3c** (IC_{50} 3.61 mg/ml)>**3d** (IC_{50} 3.69 mg/ml) and was significant ($p<0.001$) when compared to ascorbic acid (IC_{50} 0.99 mg/ml).

$$\text{OH scavenged (\%)} = \frac{(A_{\text{cont}} - A_{\text{test}})}{A_{\text{cont}}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample.

Antibacterial Activity Agar well bioassay was employed for testing antibacterial activity of **3a**—**j** (Table 1). Diluted inoculum (10^5 CFU/ml) of bacteria was spread on nutrient agar plates. Wells in the agar medium were punched and filled with the title compounds at concentration of 50 and 100 μg in each well. The plates were incubated for 24 h at 37 °C for test bacteria. The antimicrobial activity was evaluated by measuring the zone of inhibition against test organisms. Chloramphenicol was used as standard. Controls were maintained with dimethylsulphoxide (DMSO).[24]

Minimum inhibitory concentration (MIC) was determined for the compounds **3a**—**j** (Table 2) that showed total growth inhibition using the protocol described below. The compound concentration of 50 μg to 700 μg/ml in steps of 25 μg/ml was evaluated. Specifically 0.1 ml of standardized inoculum (1—2×10^7 CFU/ml) was added to each test tube. Two controls (DMSO with bacteria and antibiotics with bacteria) were maintained for each test sample. The tubes were incubated aerobically at 37 °C for 24 h.[25]

Acknowledgements The authors thank BRNS, Department of Atomic Energy (DAE), Govt. of India, Mumbai for providing financial assistance (2006/37/39/BRNS/2292).

References

1) Eto M., "Organophosphorus Pesticides, Organic and Biological Chemistry," CRC Press, Ohio, 1974, p. 152.
2) Yuksek H., Demibas A., Ikizler A., Johansson C. B., Celik C., Ikizler A. A., *Arzneim. Forsch./Drug Res.*, **47**, 405—409 (1997).
3) Ikizler A. A., Demirbas A., Johansson C. B., Celik C., Serdar M., Yuksek H., *Acta Pol. Pharm. Drug Res.*, **55**, 117—123 (1998).
4) Alkan M., Yuksek H., Islamoglu F., Bahceci S., Calapoglu M., Elmastas M., Aksit H., Ozdemir M., *Molecules*, **12**, 1805—1816 (2007).
5) Bhat A. R., Bhat G. V., Shenoy G. G., *J. Pharm. Pharmacol.*, **53**, 267—272 (2001).
6) Yuksek H., Alkan M., Akmak I., Ocak Z., Bahceci S., Calapoglu M., Elmastas M., Kolomuc A., Aksu H., *Int. J. Mol. Sci.*, **9**, 12—32 (2008).
7) Yuksek H., Kucuk M., Alkan M., Bahceci S., Kolayli S., Ocak Z., Ocak U., Sahinbas E., Ocak M., *Asian J. Chem.*, **18**, 539—550 (2006).
8) Yuksek H., Kolayli S., Kucuk M., Yuksek M. O., Ocak U., Sahinbas E., Sivrikaya E., Ocak M., *Indian J. Chem.*, **45B**, 715—718 (2006).
9) Siva Kumar B., Ravi Sankar A. U., Chandra Sekhar Reddy G., Narayana Reddy M. V., Devendranath Reddy C., Suresh Reddy C., *ARKIVOC*, **Xii**, 109—116 (2008)
10) Hussain H. H., Babic G., Durst T., Wright J., Flueraru M., Chichirau A., Chepelev L. L., *J. Org. Chem.*, **68**, 7023—7032 (2003).

11) Mc Clements J., Decker, *J. Food Sci.*, **65**, 1270—1282 (2000).
12) Gogoi P. C., Kataky C. S., *Heterocycles*, **32**, 269—272 (1991).
13) Naidu M. S. R., Raju C. N., *Indian J. Chem.*, **27B**, 88 (1988).
14) Silverstein R. M., Bassler G. C., Morrill T. C., "Spectrometric Identification of Organic Compounds," Vol. 4, John Wiley & Sons, New York, 1981.
15) Quin L. D., Verkade J. G., "Phosphorus-31 NMR Spectral Properties Compound Characterization and Structural Analysis," VCH Publishers, Inc., New York, 1994.
16) Blois M. S., *Nature* (London), **181**, 1199—1200 (1958).
17) Duh P. D., Tu Y. Y., Yen G. C., *Leben. Wissen. Technol.*, **32**, 269 (1999).
18) Yen G. C., Chen H. Y., *J. Agric. Food Chem.*, **46**, 849—854 (1995).
19) Buege J., Aust D. S., "Methods in Enzymology," Vol. 52, ed. by Fleisscher S., Packer L., Academic Press, New York, 1978, pp. 302—310.
20) Okhawa H., Ohishi N., Yagi K., *Anal. Biochem.*, **95**, 351—358 (1979).
21) Halliwell B., Gutteridge J. M. C., "Free Radicals Ageing and Disease, Free Radicals in Biology and Medicine," 2nd ed., Clarendron Press, Oxford, 1985, pp. 279—315.
22) Halliwell B., *Am. J. Med.*, **91**, 14S—22S (1991).
23) Miyake T., Shibamoto T., *J. Agric. Food Chem.*, **45**, 1819—1822 (1997).
24) Mangte D. V., Deshmukh S. P., Bhokare D. D., Deshponde Arti R., *Indian J. Pharm.*, **69**, 295 (2007).
25) Omer E., *Biologia*, **61**, 275 (2006).