Full Paper

Synthesis and Antioxidant Activity of Substituted-1,3,2-Diazaphosphole 1-Oxides

Kalla Reddi Mohan Naidu[1], Pasupuleti Visweswara Rao[2], Chamarthi Naga Raju[1], and Kambam Srinivasulu[3]

[1] Department of Chemistry, Sri Venkateswara University, Tirupati, India
[2] Department of Biotechnology, Sri Venkateswara University, Tirupati, India
[3] Department of Nano Materials Division, Graduate School of Science and Technology, Shizuoka University, Hamamatsu 432-8561, Japan

Synthesis of 1-substituted-1,3,2-diazaphosphole 1-oxides (**3a–l**) were accomplished via a two-step process. It involves the preparation of diazaphospholo 1-oxide monochloride intermediate (**2**) and its subsequent reaction with phenols/amino acid esters in dry THF in the presence of triethylamine at 40–45°C. The structures of newly synthesized compounds were characterized by spectral and elemental analysis. The title compounds were evaluated for their *in-vitro* antioxidant properties.

Keywords: Antioxidant activity / 1,3,2-Diazaphosphole 2-oxides

Received: September 26, 2010; Revised: December 16, 2010; Accepted: December 28, 2010

DOI 10.1002/ardp.201000280

Introduction

Organophosphorus heterocycles have received much attention due to their unique structural features and diverse applications in biological systems [1] particularly as possible pharmaceuticals [2]. There has been increasing interest in the role played by oxygen-derived free radicals such as the superoxide ($O_2^{\bullet}$), nitric oxide ($NO^{\bullet}$), hydroxyl ($^{\bullet}OH$) and peroxyl ($RO_2^{\bullet}$) radicals in human diseases including atherosclerosis, rheumatoid arthritis and carcinogenesis [3].

Peroxyl radicals are formed during lipid oxidation chain reactions. Lipidperoxidation may be initiated by any species that possesses sufficient reactivity to abstract a hydrogen atom from a polyunsaturated fatty acid side chain in membrane lipids [4]. Focus is made on the detailed mechanism of antioxidant action of organophosphorus heterocyclic compounds and their structure-activity relationship. Phosphite and phosphonates act as both primary and secondary antioxidants [5, 6]. 2-Substituted-thiadiazaphosphol-2-ones exhibited promising antioxidant properties [7]. Various substituted benzene-1,4-diamine-bis-dioxaphosphepine-6λ^5-iminophosphoranes showed good antioxidant activities [8]. Another series of diazaphospholoiminophosphorane derivatives containing zidovudine displayed significant antioxidant properties [9]. In view of these reports and various applications of organophosphorus heterocycles, we herein report the synthesis of a series of novel organophosphorus heterocycles by incorporating the bioactive groups attached to phosphorus atom, which fortunately resulted in noticeable antioxidant properties.

Results and discussion

Cyclocondensation of 2(±)aminomethylpiperidine (**1**) with phosphorus oxychloride in the presence of TEA in dry THF at 10–20°C gave monochloride **2** which on subsequent reaction with phenols/amino acid esters yielded **3a–l**. This method is advantageous since it does not require the preparation of highly toxic, moisture sensitive and thermally unstable aryl phosphorodichlorides of the corresponding phenols and amino acid esters (Scheme 1). The cyclized products (**3a–l**) were isolated by filtration to remove TEA hydrochloride, followed by evaporation of the filtrate in a rotary evaporator. Further purification was carried out by washing the residue with hexane followed by column chromatography using hexane/ethyl acetate (8:2) mixture as an eluent.

All the compounds **3a–l** exhibited IR absorption bands for P=O and P-NH in the regions 1269–1214 and 3410–3148 cm^{-1} [10], respectively. The –NH proton gave a singlet at δ 3.55–2.42,

Correspondence: Chamarthi Naga Raju, Department of Chemistry, Sri Venkateswara University, Tirupati-517502, India.
E-mail: rajuchamarthi10@gmail.com
Fax: +91877 222521

the C-3 methylene hydrogens resonated as multiplets at δ 3.40–2.21. The N–CH proton is also appeared as a multiplet at δ 3.68–2.92. In the ^{13}C-NMR spectra, C-3 methylene resonated at δ 58.3–45.8. The chemical shifts of the α-carbon to the amino acid ester group appeared at δ 59.8–52.6 [11]. The remaining carbon resonances were observed in the expected region. ^{31}P-NMR signals were observed in the region 26.19–14.30 ppm [12]. The APCI-MS data of all the products showed their protonated molecular ions.

Antioxidant activity

Results on the preliminary antioxidant activity of the title compounds are presented in Table 1. The compound **3g** with a L-valine methylester substitution at phosphorus atom of octahydro-1,3,2-diazaphospholo[1,5a]pyridine-1-oxide showed enhanced antioxidant activity by maximum levels of enzymes like catalase, lipidperoxidation, and glutathione. Whereas **3g** showed a little reduction in superoxide dismutase activity and the compound **3l** having a substitution of phenylglycine ethylester at phosphorus atom of the title compound exhibited an enhanced superoxide dismutase activity, followed by catalase activity. But the studies showed no enhancement of LPO and GSH activities with administration of the compound **3l**. Further observations reveal that the compounds **3g**, **3h**, **3k**, and **3l** with corresponding amino acid esters as substituents showed a clear enhancement in the levels of antioxidant enzyme activities. The compound **3f** with a *bis*-(2-chloro) ethyl amine substitution showed an enhanced antioxidant activity as reported earlier [13].

Conclusion

Synthesis of a series of novel 1-substituted-1,3,2-diazaphosphole-1-oxides is accomplished by adopting a simple and straight forward synthetic protocol. The structures of **3a–l** were established by elemental analysis, IR, NMR (^{1}H-, ^{13}C-, and ^{31}P-), and mass spectral data. Antioxidant properties were evaluated for the title compounds (**3a–l**), the compounds **3g** exhibited good antioxidant property when compared to other members of the series except against SOD. The compound **3l** showed maximum antioxidant activity against SOD. Overall, the title compounds exhibited moderate antioxidant properties when compared to that of vitamin C.

Experimental

Chemistry

Chemicals were purchased from Sigma-Aldrich, Merck and Lancaster, and were used as such without further purification. All solvents used for spectroscopic and other physical studies were reagent grade and were further purified by literature methods [14]. Melting points were determined using a calibrated thermometer by Guna Digital Melting point apparatus. 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 a Bruker 400 MHz NMR spectrometer operating at 500 MHz for ^{1}H-, 125 MHz for ^{13}C-, and 202 MHz for ^{31}P-NMR. Spectra were recorded in DMSO-d_6 and referenced to TMS (^{1}H & ^{13}C) and 85% H_3PO_4 (^{31}P). APCI mass spectra were recorded on a Jeol SX102 DA/600 mass spectrometer. Elemental analyses were performed on a Thermo Finnigan Insturment at University of Hyderabad, Hyderabad, India.

*Synthetic procedure for the intermediate (**2**)*

A solution of phosphoryl chloride (0.002 mol) in 15 mL of dry THF was added dropwise over a period of 15 min to a stirred solution of 2(±)-aminomethyl piperidine (**1**) (0.002 mol) and triethylamine (0.004 mol) in 10 mL of THF at 0–5°C. After stirring for 1 h at room temperature, formation of intermediate monochloride **2** was ascertained by TLC analysis run in a 3:7 mixture of ethyl acetate and hexane, TEA hydrochloride was removed from the reaction mixture by filtration. The filtrate is evaporated in a

Table 1. Antioxidants in brain homogenates of young rats using test compounds **3a–l**.

Parameters	SOD	CAT	LPO	GSH
3a	26.23 ± 0.27	262.21 ±6 .23	69.71 ± 3.15	4.72 ± 0.74
3b	26.51 ± 1.47	259.63 ± 4.17	68.02 ± 1.31	4.61 ± 1.56
3c	25.87 ± 1.62	260.08 ± 5.22	66.36 ± 1.45	4.52 ± 1.28
3d	25.91 ± 1.43	261.92 ± 5.76	69.19 ± 2.38	4.67 ± 1.90
3e	26.18 ± 1.39	260.34 ± 6.23	67.22 ± 1.10	4.62 ± 1.33
3f	27.62 ± 1.21	264.25 ± 6.23	72.39 ± 1.66	4.81 ± 0.66
3g	27.29 ± 0.55	278.16 ± 4.61	77.16 ± 1.52	4.95 ± 1.51
3h	27.93 ± 1.88	272.13 ± 5.78	71.44 ± 1.57	4.83 ± 1.73
3i	25.72 ± 1.94	263.11 ± 4.32	62.48 ± 2.77	4.59 ± 1.29
3j	26.23 ± 1.46	267.18 ± 5.22	65.62 ± 1.33	4.64 ± 1.50
3k	27.26 ± 1.32	272.88 ± 2.18	76.22 ± 1.28	4.71 ± 1.07
3l	28.44 ± 0.71	273.02 ± 5.76	72.11 ± 1.82	4.68 ± 1.55
Ascorbic acid	32.45 ± 1.25	385.46 ± 5.25	82.78 ± 1.43	8.34 ± 0.74
Vitamin E	34.06 ± 1.28	403.27 ± 4.76	88.06 ± 1.28	10.26 ± 0.92

Abbreviations: LPO – lipid peroxidation; GSH – reduced glutathione content; SOD – superoxide dismutase; CAT – catalase.

rotary evaporator to get the intermediate (**2**). The intermediate was confirmed by ^{1}H-, ^{13}C-, ^{31}P-NMR and mass spectral analyses.

1-Chloro-hexahydro-[1,3,2]diazaphospholo[1,5a]-pyridine-1-oxide ***2***

^{1}H-NMR ($CDCl_3$): 3.59–3.49 (1H, m, N-CH), 3.45–3.42 (2H, m, NH-CH_2), 2.26 (1H, s, NH), 1.89–1.84 (2H, m, N–CH_2), 1.74–1.68 (6H, m, –$(CH_2)_3$); ^{13}C-NMR data: 66.6 (C-4), 44.8 (C-3), 44.3 (C-6), 30.3 (C-9), 29.6 (C-7), 26.6 (C-8); ^{31}P-NMR: δ 22.0; APCI-MS *m/z* (%) 194 [M^+] (100), 196 [M + 2] (32).

*Synthetic procedure for the title compounds (**3a–l**)*

To a stirred solution of various phenols and amino acid ester hydrochlorides in dry THF (10 mL) and TEA (0.002 mol), the intermediate monochloride (**2**) in dry THF was added dropwise at 0°C. After the addition, the temperature was slowly raised to 40–45°C and the mixtures were stirred for 2 h. The progress of the reaction was monitored by TLC conducted on a 3:7 mixture of ethylacetate and hexane with an average R_f value 0.65. The reaction mixtures were filtered to remove TEA hydrochloride and the filtrate on evaporation in a rotary evaporator yielded the crude products. These were further purified by column chromatography on silica gel (60–120 mesh) with ethyl acetate/hexane (1:9) as eluent. The title compounds were characterized by IR, ^{1}H-, ^{13}C-, ^{31}P-NMR and mass spectral analyses.

1-(4-Chlorophenoxy)-hexahydro-[1,3,2]-diazaphospholo[1,5a]pyridine-1-oxide ***3a***

Yield 80%; m.p. 127–29°C; IR (KBr) (ν_{max}, cm^{-1}): 3315 (–NH), 1250 (P=O), 1210, 950 (P–O–C_{aryl}) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 7.41 (2H, d, J = 7.8 Hz, Ar-H), 7.29 (2H, d, J = 7.4 Hz, Ar-H), 3.34–3.03 (1H, m, N-CH), 2.96–2.93 (2H, m, NH-CH_2), 2.74–2.62 (2H, m, N–CH_2), 2.51 (1H, s, NH), 1.56–1.16(6H, m, –$(CH_2)_3$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 151.2 (C-1′), 129.3 (C-3′, C-5′), 124.0 (C-4′), 122.2 (C-2′, C-6′), 55.6 (C-4), 45.8 (C-3), 44.7 (C-6), 30.7 (C-9), 28.0 (C-7), 23.4 (C-8); ^{31}P-NMR: δ 22.0; APCI-MS *m/z* (%) 286 [M^+] (100), 288 [M + 2] (32), 287.3 [M + H]$^+$ (13.2); Anal. calcd. for $C_{12}H_{16}ClN_2O_2P$: C, 50.26, H, 5.58, N, 9.77. Found: C, 50.21, H, 5.53, N, 9.85.

1-(4-Nitrophenoxy)-hexahydro-[1,3,2]-diazaphospholo[1,5a]pyridine-1-oxide ***3b***

Yield 82%; m.p. 135–37°C; IR (KBr) (ν_{max}, cm^{-1}): 3275 (-NH), 1263 (P=O), 1212, 968 (P–O–C_{aryl}) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 8.11 (2H, d, J = 9.0 Hz, Ar-H), 6.92 (2H, d, J = 9.0 Hz, Ar-H), 3.30–3.25 (1H, m, N–CH), 2.89–2.74 (2H, m, NH–CH_2), 2.61 (1H, s, NH), 2.59–2.54 (2H, m, N–CH_2), 1.27–1.13 (6H, m, –$(CH_2)_3$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 164.3 (C-1′), 126.3 (C-3′, C-5′), 140.6 (C-4′), 116.4 (C-2′, C-6′), 55.1 (C-4), 48.9 (C-3), 43.2 (C-6), 31.7 (C-9), 29.4 (C-7), 22.8 (C-8); ^{31}P-NMR: δ 22.83; APCI-MS *m/z* (%) 298 [MH]$^+$ (100); Anal. calcd. for $C_{12}H_{16}N_3O_4P$: C, 48.48, H, 5.38, N, 14.14. Found: C, 48.51, H, 5.42, N, 14.17.

1-(Pyridin-3-yloxy)-hexahydro-[1,3,2]diazaphospholo-[1,5a]pyridine-1-oxide ***3c***

Yield 78%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3248 (–NH), 1260 (P=O), 1217, 958 (P–O–C_{aryl}) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 7.41–7.29 (4H, m, Ar-H), 3.34–3.03 (1H, m, N–CH), 2.96–2.90 (2H, m, NH–CH_2), 2.62–2.53 (2H, m, N–CH_2), 2.51 (1H, s, NH), 1.56–1.16 (6H, m, –$(CH_2)_3$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 160.2 (C-2′), 149.5 (C-1′), 139.1 (C-4′), 121.1 (C-5′), 120.4 (C-6′), 54.9 (C-4), 45.3 (C-3), 43.7 (C-6), 29.8 (C-9), 27.8 (C-7), 23.2 (C-8); ^{31}P-NMR: δ 24.20; APCI-MS *m/z* (%) 255 [M + 2] (55); Anal. calcd. for $C_{11}H_{16}N_3O_2P$: C, 52.17, H, 6.32, N, 16.9. Found: C, 52.24, H, 6.37, N, 17.2.

1-Propyl-hexahydro-[1,3,2]diazaphospholo[1,5a]-pyridine-1-oxide ***3d***

Yield 76%; m.p. 126–128°C IR (KBr) (ν_{max}, cm^{-1}): 3368 (-NH), 1269 (P=O) cm^{-1}; ^{1}H-NMR (MEOD) δ [ppm]: 3.53–3.41 (1H, m, N–CH), 3.05–3.01 (2H, m, NH–CH_2), 2.65–2.53 (2H, m, N–CH_2), 2.42 (1H, s, NH), 1.65–1.50 (6H, m, –$(CH_2)_3$), 1.51–1.44 (4H, m, –$(CH_2)_2$–), 0.93 (3H, t, J = 6.5 Hz, –CH_3); ^{13}C-NMR (MEOD) δ [ppm]: 60.9 (C-6), 58.3 (C-3), 44.1 (C-4), 30.4 (C-9), 26.5 (C-7), 22.3 (C-1′), 22.1 (C-8), 17.3 (C-2′), 15.9 (C-3′); ^{31}P-NMR: δ 26.19; APCI-MS *m/z* (%) 203 [MH]$^+$ (100); Anal. calcd. for $C_9H_{19}N_2OP$: C, 53.46, H, 9.40, N, 13.86. Found: C, 53.38, H, 9.45, N, 13.92.

1-Phenyl-hexahydro-[1,3,2]diazaphospholo[1,5a]-pyridine-1-oxide ***3e***

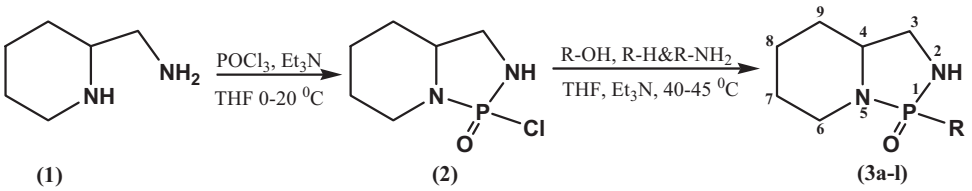
Yield 81%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3148 (-NH), 1262 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 7.75–7.27 (5H, m, Ar-H), 3.68–3.61 (1H, m, N–CH), 2.95–2.86 (2H, m, NH–CH_2), 2.71–2.65 (2H, m, N–CH_2), 2.55 (1H, s, NH), 1.59–1.30 (6H, m, –$(CH_2)_3$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 130.4 (C-3′, C-5′), 129.5 (C-4′), 127.5 (C-2′, C-6′), 129.6 (C-1′), 58.2 (C-4), 46.1 (C-3), 42.2 (C-6), 30.5 (C-9), 29.0 (C-7), 21.6 (C-8); ^{31}P-NMR: δ 22.83; APCI-MS *m/z* (%) 237 [MH] $^+$ (100); Anal. calcd. for $C_{12}H_{17}N_2OP$: C, 61.01, H, 7.17, N, 11.81. Found: C, 61.06, H, 7.21, N, 11.87.

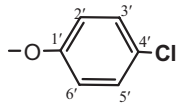
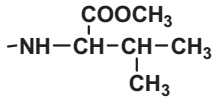
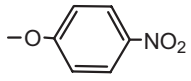
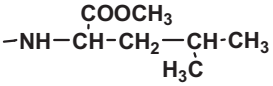
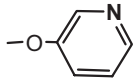
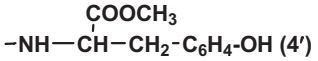
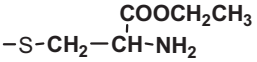
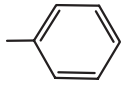
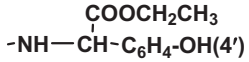
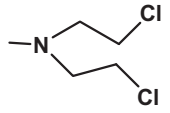
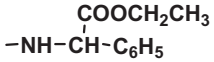
N,N-bis(2-chloroethyl)-hexahydro-[1,3,2]-diazaphospholo[1,5a]pyridine-1-amine 1-oxide ***3f***

Yield 79%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3367 (-NH), 1233 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.19–3.85 (4H, m, –CH_2–Cl), 3.68–3.54 (1H, m, N–CH), 3.40–3.35 (4H, m, N–CH_2), 2.85–2.72 (2H, m, NH–CH_2), 2.62–2.54 (2H, m, N–CH_2), 2.47 (1H, s, NH), 1.89–1.16 (6H, m, –$(CH_2)_3$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 64.5 (C-4), 53.8 (C-1′), 48.1 (C-3), 47.0 (C-6), 43.2 (C-2′), 30.5 (C-9), 26.2 (C-7), 21.8 (C-8); ^{31}P-NMR: δ 26.02; APCI-MS *m/z* (%); 299 [M^+] (100), 301 [M + 2] (65), 303 [M + 4] (8.3); Anal. calcd. for $C_{10}H_{20}Cl_2N_3OP$: C, 40.01, H, 6.66, N, 14.02. Found: C, 40.12, H, 6.61, N, 14.08.

Methyl-2-methyl-1-[(1-oxido-hexahydro-[1,3,2]-diazaphospholo[1,5a]pyridine-1-yl)amino] butanoate ***3g***

Yield 82%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3426 (-NH), 3152 (-NH), 1721 (C=O), 1204 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 3.82 (3H, s, –OCH_3), 3.75–3.68 (1H, m, NH–CH–CO), 3.65–3.60 (1H, m, N–CH), 3.55 (2H, s, NH), 2.71–2.64 (2H, m, N–CH_2), 2.35–2.21 (2H, m, NH–CH_2), 1.55–1.44 (6H, m, –$(CH_2)_3$), 1.37–1.26 (1H, m, –CH–$(CH_3)_2$), 1.13 (6H, d, J = 6.5 Hz, –CH–$(CH_3)_2$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 165.3 (C-5′), 61.6 (C-4), 59.8 (C-1′), 55.8 (C-3), 52.7 (C-6′), 45.8 (C-6), 41.5 (C-2′,), 30.7 (C-9), 27.8 (C-7), 23.5 (C-8), 18.3 (C-3′, C-4′); ^{31}P-NMR: δ 23.10; APCI-MS *m/z* (%) 290 [MH]$^+$ (60); Anal. calcd. for $C_{12}H_{24}N_3O_3P$: C, 49.82, H, 8.30, N, 14.53. Found: C, 49.89, H, 8.34, N, 14.57.



Compd	R	Compd	R
3a		3g	
3b		3h	
3c		3i	
3d	-CH_2-CH_2-CH_3	3j	
3e		3k	
3f		3l	

Scheme 1. Synthesis of title compounds **3a–l.**

Methyl-3-methyl-1-[(1-oxido-hexahydro-[1,3,2]-diazaphospholo[1,5a]pyridine-1-yl)amino] pentanoate ***3h***

Yield 79%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3259 (–NH), 3152 (–NH), 1740 (C=O), 1230 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 3.80 (3H, s, $-OCH_3$), 3.70–3.65 (1H, m, NH–CH–CO), 3.45–3.32 (1H, m, N–CH), 3.32 (2H, s, NH), 3.01–2.96 (2H, m, N–CH_2), 2.30–2.24 (2H, m, NH–CH_2), 1.60–1.45 (6H, m, $-(CH_2)_3$), 1.37–1.26 (1H, m, $-CH-(CH_3)_2$), 1.15 (6H, d, J = 6.5Hz, $-CH-(CH_3)_2$), 1.89 (2H, t, J = 8.2 Hz, CH–CH_2–CH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 169.6 (C-6′), 55.5 (C-4), 54.8 (C-7′), 53.4 (C-3), 52.6 (C-1′), 45.7 (C-6), 41.7 (C-2′), 30.6 (C-9), 27.7 (C-7), 25.8 (C-3′), 23.5 (C-8), 22.6 (C-5′, C-4′); ^{31}P-NMR: δ 22.93; APCI-MS m/z (%) 304 $[MH]^+$ (60); Anal. calcd. for $C_{13}H_{26}N_3O_3P$: C, 51.48, H, 8.58, N, 13.86. Found: C, 51.56, H, 8.61, N, 13.90.

Methyl-2(4-hydroxyphenyl)-1-[(1oxido-hexahydro-[1,3,2]-diazaphospholo[1,5a]pyridine-1-yl)amino] propanoate ***3i***

Yield 76%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3512 (–OH), 3326 (–NH), 3162 (–NH), 1746 (C=O), 1214 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 10.10 (1H, s, Ar–OH), 7.81 (2H, d, J = 9.0 Hz, Ar–H), 6.92 (2H, d, J = 9.0 Hz, Ar-H), 3.82 (3H, s, $-OCH_3$), 3.72–3.64 (1H, m, NH–CH–CO), 3.52–3.45 (1H, m, N–CH), 3.37 (2H, s, NH), 3.05–2.94 (2H, m, N–CH_2), 2.86–2.74 (2H, m, NH–CH_2), 2.6 (2H, d, J = 6.6 Hz, Ar–CH_2–CH–), 1.80–1.40 (6H, m, $-(CH_2)_3$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 168.5 (C-9′), 151.4 (C-6′), 129.5 (C-4′, C-8′), 117.4 (C-5′, C-7′), 56.2 (C-4), 52.4 (C-10′), 50.2 (C-1′), 49.0 (C-3), 45.6 (C-6), 39.0 (C-2′), 28.5 (C-9), 25.2 (C-7), 22.1 (C-8); ^{31}P-NMR: δ 21.17; APCI-MS m/z (%) 354 $[MH]^+$ (60); Anal. calcd. for $C_{16}H_{24}N_3O_4P$: C, 52.78, H, 7.03, N, 12.31. Found: C, 52.84, H, 7.09, N, 12.35.

Ethyl-2-amino-1-[(1-oxido-hexahydro-[1,3,2]-diazaphospholo[1,5a] pyridine-1-yl)sulfonyl] propanoate ***3j***

Yield 78%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3516 (-OH), 3372 (–NH), 3182 (–NH), 1735 (C=O), 1231 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 3.72 (2H, s, $-OCH_2$), 3.03–2.92 (1H, m, N–CH), 3.37 (2H, s, NH_2), 2.85–2.76 (2H, m, NH–CH_2), 2.67–2.52 (2H, m, N–CH_2), 2.42 (1H, s, –NH), 1.95–1.83 (2H, s, S–CH_2), 1.52–1.34 (6H, m, $-(CH_2)_3$), 1.12 (3H, t, J = 6.5 Hz, $-CH_3$), 3.19–3.04 (1H, m, $-CH-NH_2$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 169.9 (C-3′), 61.1 (C-4′), 55.8 (C-4), 49.4 (C-3), 41.7 (C-1′), 45.2 (C-6), 42.6 (C-2′), 30.1 (C-9), 28.7 (C-7), 22.5 (C-8), ^{31}P-NMR: δ 22.12; APCI-MS m/z (%) 308 $[MH]^+$ (60); Anal. calcd. for $C_{11}H_{22}N_3O_3PS$: C, 42.99, H, 7.16, N, 13.68. Found: C, 43.05, H, 7.21, N, 13.72.

Ethyl (4-hydroxyphenyl)-1-[(1-oxido-hexahydro-[1,3,2]-diazaphospholo[1,5a]pyridine 1-yl)amino]acetate ***3k***

Yield 80%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3514 (–OH), 3326 (–NH), 3182 (–NH), 1738 (C=O), 1265 (P=O) cm^{-1}; ^{1}H–NMR (DMSO-d_6) δ [ppm]: 9.90 (1H, s, Ar–OH), 7.12 (2H, d, J = 9.0 Hz, Ar–H), 6.76 (2H, d, J = 9.0 Hz, Ar–H), 3.80 (2H, s, $-OCH_2$), 3.89–3.74 (1H, m, NH–CH), 3.47–3.30 (1H, m, N–CH), 3.32 (1H, s, NH), 2.96–2.82 (2H, m, N–CH_2), 2.89–2.72 (2H, m, NH–CH_2), 1.52–1.36 (6H, m, –$(CH_2)_3$), 1.12 (3H, t, J = 7.0 Hz, $-CH_3$); ^{13}C–NMR (DMSO–d_6) δ [ppm]: 167.8 (C-8′), 151.7 (C-5′), 128.5 (C-3′, C-7′), 127.2 (C-2′), 115.2 (C-4′, C-6′), 62.2 (C-9′), 56.4 (C-4), 51.8 (C-1′), 48.6 (C-3), 44.9 (C-6), 29.2 (C-9), 24.6 (C-7), 23.4 (C-8), 16.2 (C-10′); ^{31}P-NMR: δ 23.02; APCI-MS m/z (%) 354[MH]$^+$ (60); Anal. calcd. for $C_{16}H_{24}N_3O_4P$: C, 54.39, H, 6.79, N, 11.89. Found: C, 54.46, H, 6.82, N, 11.94.

Ethyl-[(1-oxido-hexahydro-[1,3,2]diazaphospholo[1,5a]-pyridine-1-yl)amino]phenyl acetate ***3l***

Yield 81%; viscous liquid, IR (KBr) (ν_{max}, cm^{-1}): 3410 (–NH), 3172 (–NH), 1728 (C=O), 1237 (P=O) cm^{-1}; ^{1}H–NMR (DMSO–d_6) δ [ppm]: 7.29–6.31 (5H, m, Ar–H), 3.92–3.85 (1H, m, NH–CH), 3.71 (2H, s, $-OCH_2$), 3.32–3.26 (1H, m, N–CH), 2.85–2.71 (2H, m, NH–CH_2), 2.92–2.84 (2H, m, N–CH_2), 2.55 (2H, s, NH), 1.57–1.31 (6H, m, –$(CH_2)_3$), 1.15 (3H, t, J = 7.5 Hz, $-CH_3$); ^{13}C–NMR (DMSO–d_6) δ [ppm]: 169.4 (C-8′), 133.2 (C-2′), 128.2 (C-3′, C-7′), 127.9 (C-4′, C-6′), 125.2 (C-5′), 61.7 (C-9′), 55.6 (C-4), 51.4 (C-1′), 46.6 (C-3), 44.2 (C-6), 28.1 (C-9), 25.4 (C-7), 22.1 (C-8), 16.6 (C-10′); ^{31}P-NMR: δ 14.53; APCI-MS m/z (%) 338 [MH]$^+$ (100); Anal. calcd. for $C_{16}H_{24}N_3O_3P$: C, 56.97, H, 7.12, N, 12.46. Found: C, 57.03, H, 7.16, N, 12.51.

Antioxidant acitiivty [13]

Adult Wistar albino rats (90 days) were used in the study. The rats were fed a standard pellet diet (Hindustan Lever Ltd., India) and were given water *ad libitum*. The animals were maintained under proper temperature (25–30°C), ventilation, and hygienic conditions. They were exposed to 12 h each of light and dark. The title compounds **3a-l** were used to estimate the antioxidant activity *in vitro*. The oxidative stress was assessed by estimating lipid peroxidation (LPO), reduced glutathione content (GSH), the activities of superoxide dismutase (SOD), and catalase (CAT). The results were compared with that of vitamin C, the known natural antioxidant. The formula for calculation of antioxidant activities for LOP, SOD, GSH, CAT are as follows:

- Inhibition of lipid peroxidation:

$$\%\,\text{Inhibition} = \frac{100 \times A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}$$

- Superoxide dismutase activity:

$$\text{Superoxide dismutase} = \frac{0.93 \times (V_s/V_c) - 1}{1.073 - 0.073\,(V_s/V_c)}$$

where V_s is the rate of sample containing SOD and V_c is the average rate of blank sample (SOD = 0)

- Glutathione content:

$$\text{GSH} = \frac{\text{Net rate} - \text{Intercept}}{\text{Slope}} \pm \text{Dilution factor}$$

- Catalase activity:

$$\text{CAT} = \frac{\text{OD of the sample} \times \text{Volume of assay}}{13.6 \times \text{Volume of enzyme source} \times \text{mg of protein}}$$

where OD = optical density

Brain tissues of rat were homogenized 1:40 (w/v) in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. Total glutathione (GSH + GSSG) [15] and reduced glutathione (GSH) and the activities of GSH-PX [16] and GST [17] were assayed. Protein content of the homogenates was determined by the method of Lowry et al. [18] and the GSH-PX and GST activities were expressed in terms of U/g protein. For estimation of ascorbic acid the tissue were homogenized 1:9 (w/v) in ice-cold 5% trichloroacetic acid. Ascorbic acid assay is based on oxidation of ascorbic acid to dehydroascorbic acid followed by treatment with 2,4-dinitrophenylhydrazine to form the *bis*-dinitrophenylhydrazone [19].

The authors express their grateful thanks to UGC, New Delhi, for sanctioning a major Research Project (F.No. 34–356/2008(SR) and Prof. C. D. Reddy, Department of Chemistry, Sri Venkateswara University, Tirupati, for his helpful discussion.

The authors have declared no conflict of interest.

References

[1] F. H. Westheimer, *Science* **1987**, *235*, 1173–1178.

[2] P. Kafarski, B. Lejczak, *Curr. Med. Chem.* **2001**, *1*, 301–312.

[3] C. Rice-Evans, A. T. Diplock, *Techniques in Free Radical Research*, Elsevier, Amsterdam **1991**, p. 291.

[4] H. A. Kappus, *Chem. Phys. Lipids* **1987**, *45*, 105–115.

[5] K. Schwetlick, in *Mechanism of Polymer Degradation and Stabilisation* (Ed.: G. Scott), Elsevier Applied Science, London and New York **1990**, p. 23.

[6] K. Schwetlick, *Pure Appl. Chem.* **1983**, *55*, 1629–1636.

[7] A. Balakrishna, M. V. N. Reddy, S. K. Naik, M. Manjunath, C. D. Reddy, C. Suresh Reddy, *Chem. Pharm. Bull.* **2008**, *56*, 1486–1489.

[8] M. V. N. Reddy, G. Chandrasekhar Reddy, K. Suresh Kumar, C. Suresh Reddy, C. Naga Raju, *J. Heterocycl. Chem.* **2010**, *47*, 538–542.

[9] A. Janardhan Rao, V. Koteswara Rao, P. Visweswara Rao, B. Sateesh Krishna, C. Naga Raju, S. K. Ghosh, *Int. J. Pharm. Biol. Sci.* **2010**, *1*, 1–10.

[10] K. R. Kishore Kumar Reddy, M. Anil Kumar, M. V. Narayana Reddy, C. Devendranath Reddy, C. Suresh Reddy, *Tetrahedron Lett.* **2008**, *49*, 6337–6340.

[11] M. Anil Kumar, K. Suresh Kumar, C. Devendranath Reddy, C. Naga Raju, C. Suresh Reddy, P. Hari Krishna, *South Afr. J. Chem.* **2009**, *62*, 26–29.

[12] R. Wang, S. Harada, H. Mitsuya, J. Zemlicka, *J. Med. Chem.* **2003**, *22*, 4799–4802.

[13] V. Koteswara Rao, A. Janardhan Rao, S. Subba Reddy, C. Naga Raju, P. Visweswara Rao, S. K. Ghosh, *Eur. J. Med. Chem.* **2010**, *45*, 203–209.

[14] W. L. F. Armarego, D. D. Perrin, *Purification of Laboratory Chemicals*, 4th Ed, Butterworth, Heinemann, Oxford **1997**.

[15] E. Beutler, O. Duron, B. M. Kelly, *J. Lab. Clin. Med.* **1963**, *61*, 882.

[16] A. Wendel, Glutathione peroxidase, in *Methods in Enzymology* (Ed.: W. B. Jakoby), Vol. *77*, Academic Press, New York **1981**, p. 325.

[17] W. H. Habig, M. J. Pabst, W. B. Jakoby, *J. Biol. Chem.* **1974**, *249*, 7130–7139.

[18] O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **1951**, *193*, 265–275.

[19] S. T. Omaye, J. D. Turnbull, H. E. Säuberlich, Selected Methods for the Determination of Ascorbic Acid in Animal Cells, Tissues and Fluids, in *Methods in Enzymology* (Eds: D. B. MaCormick, L. D. Wright), Vol. *62*, Academic Press, New York **1979**, p. 3.

