ORIGINAL ARTICLE

A C G publications

Org. Commun. 8:1 (2015) 17-23

organic communications

Synthesis and antioxidant activity of dioxazaphosphinin-2-ones

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(Received May 2, 2014; Revised February 7, 2015; Accepted February 9, 2015)

Abstract: A simple and efficient synthesis of dioxazaphosphinin-2-ones has been developed by the cyclisation of 2-hydroxy-1,2-diphenylethanoneoxime with phosphorus oxychloride in the presence of triethylamine in dry THF to afford the corresponding monochloride. In the next step, subsequent reaction of monochloride with different amines at 50-60 °C for 2-3 hours resulted in the formation of the title compounds **(4a-j)**. The lead compounds were screened for their antioxidant activity.

Keywords: 2-hydroxy 1,2-diphenylethanoneoxime; $POCl_3$; dioxazaphosphinin-2-ones; Antioxidant activity.

1. Introduction

Six-membered phosphorus heterocycles containing O, N as heteroatoms and P as P=O or P=S have been the subject of research ever since cyclophosphamide was discovered as cancer drug.[1]

Cyclophosphamide is also used to treat severe rheumatoid arthritis,[2] Wegener's granulomatosis,[3] and multiple sclerosis.[4] The main use of cyclophosphamide is with other chemotherapy agents in the treatment of lymphomas, some forms of brain cancer, leukemia[5] and some solid tumors.[6] It is a chemotherapy drug that works by inducing the death of certain T cells.

In recent years the antioxidant efficiency of organophosphorus compounds and their relationships between chemical structure and hydrogen radical donating abilities have been related.[7,8] However, the detailed mechanism underlying the effect of phosphorus bearing moieties on the antioxidant potential has not been studied in any detail. Depending on their structure, the additional negative charge generated at phosphorus in phosphites and phosphonates can be expected to affect its radical scavenging properties. The immune system in several organisms is a sensitive target to the toxic action of reactive oxygen species (ROS). ROS are produced by univalent reduction of dioxygen to superoxide anion which in turn disproportionate to H_2O_2 and O_2 spontaneously. The ROS are believed to play a major role in the diminishing inflammatory process in rheumatoid arthritis (RA) and contribute to the destruction of cartilage and bone.[9,10]

The most important ROS implicated in inflammatory tissue injury are superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl ($HO^{\cdot}$) and hypochlorous acid ($HOCl^{\cdot}$) radicals. In the inflammed joint, these species are produced by macrophages, neutrophils and chondrocytes.[11] The inflammed rheumatoid joint also undergoes a hypoxia-reperfusion cycle which results in ROS generation.[12] Antioxidants may have a therapeutic role in RA by suppressing the inflammation.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/OC/index.htm © Published 01/03/2015 EISSN:1307-6175

Different antioxidant parameters, like the antioxidative enzymes including superoxide dismutase, glutathione peroxidase and catalase (CAT) are commonly used to assess exposure to ROS in living cells. Particularly CAT is an enzyme promoting the conversion of hydrogen peroxide (H_2O_2) to water and molecular oxygen and can be used as a biomarker for oxidative stress. A number of studies reported an increase of superoxidase dismutase and CAT activities when an excess of ROS was observed in aerobic cells.[13]

Balakrishna *et al*.[8] reported condensation of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol with various phosphorus dichlorides in the presence of triethylamine in dry tetrahydrofuran at 60-65 °C and obtained corresponding thiadiazaphosphol-2-ones. These 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 also.

A new series of 5-chloro-3-(4-substituted anilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-ones have been prepared via Mannich type reaction by reacting 5-chlorosalicyaldehyde with phenyl dichloro phosphine and aromatic amines in anhydrous benzene under reflux condition. The new benzoxaphosphol-2-one derivatives were found to possess moderate to good antioxidant and anti-microbial activity.[14]

Anil *et al*.[15] synthesized 2-(aminoacid ester)-3-(6-methyl-2-pyridyl)-3,4-dihydro-2*H*-1,3,2λ^5-benzoxazaphosphinin-2-thiones through a two-step process. It involves the prior preparation of 2-chloro-3-(6-methyl-2-pyridyl)-3,4-dihydro-2*H*-1,3,2λ^5benzoxazaphosphinin-2-thione monochloride and its subsequent reaction with the aminoacid ester hydrochlorides in dry tetrahydrofuran-toluene in the presence of triethylamine at various temperatures.

Even though several compounds related to cyclophosphamide have been synthesized, none of them was found to possess satisfactory pharmacological properties. Hence the search continued for the development of potential bioactive molecule from cyclophosphamide family. In the present investigation, a series of newly synthesized dioxazaphosphinin-2-ones were successfully synthesized and their antioxidant activity was also evaluated.

2. Results and Discussion

Syntheses of 2-substituted-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-ones **(4a-j)** were conveniently accomplished in a two-step process. The first step involves the cyclisation of 2-hydroxy 1,2-diphenylethanoneoxime **(1)** with $POCl_3$ in the presence of triethylamine in dry THF to afford the corresponding monochloride **(2)**. In the second step, subsequent reaction of **2** with different amines **(3a-j)** at 50-60 ˚C for 2-3 hours resulted in the formation of the title compounds **(Figure 1)**.

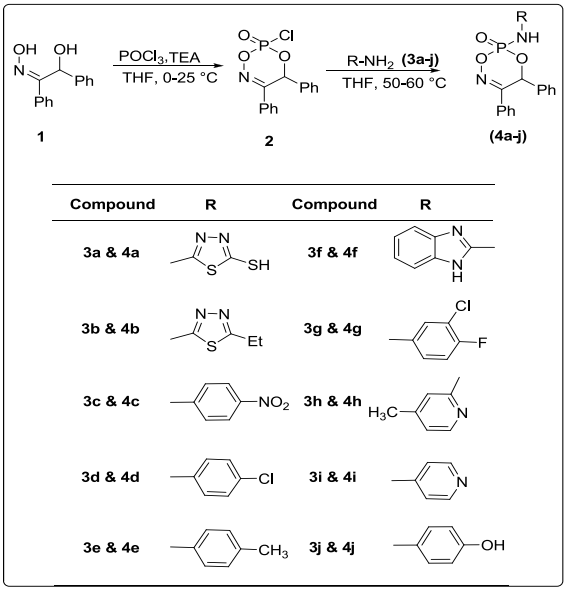

Figure 1. Synthesis of dioxazaphosphinin-2-ones (4a-j)

3. Experimental procedures

3.1. General

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.

3.2. *Typical procedure for the synthesis of* (*4d*)

To a stirred solution of 2-hydroxy 1,2-diphenylethanoneoxime **(1)** in dry tetrahydrofuran (25 mL) was added drop wise $POCl_3$ in dry tetrahydrofuran (10mL) in the presence of NEt_3 (0.004) at 0˚C. After the addition temperature of the reaction mixture was slowly raised to 50-60 ˚C. Progress of the reaction was monitored by Thin layer chromatography (TLC) analysis by using 3:7 mixture of EtOAc and hexane. The solid trietylamine hydrochloride was separated by filtrate to obtain the monochloride 2-chloro-5,6-diphenyl-6H-1,3,4,2λ^5-dioxaza-phosphinin-2-one **(2)**. This monochloride was used for the next step of the reaction without purification.

To this monochloride in THF, solution of *p*-chloroaniline in anhydrous THF (15mL) in the presence of NEt_3 was added slowly and after completion of the addition, the reaction mixture was kept stirring at 50-60 ˚C for 2-3 hours. Completion of the reaction was monitored by TLC analysis. The solid NEt_3.HCl was filtered and the filtrate was subjected to rota-evaporation under reduced pressure to obtain the crude product. It was further purified by column chromatography on silica gel (100-200 mesh) with EtOAc: Hexane (1:9) as eluent to get pure compounds which were characterized by IR, ^{1}H, ^{13}C, ^{31}P NMR and Mass spectral analysis. Same procedure was applied to the remaining compounds.

3.3. Spectral data for the compounds 4a-j

3.3.1. 5,6-diphenyl-2-[(5-sulfanyl-1,3,4-thiadiazol-2-yl)amino]-6H-1,3,4,2λ^5-dioxazaphosphinin- 2 one (4a):
Yield: 89%, mp: 222-223 ºC. IR (KBr) cm^{-1}: 3388(NH), 1727(P=O), 1679(C=N), 1174 (P-N). ^{1}H NMR δ: 7.12-8.20 (m, 10H), 4.08 (s, 1H), 3.82 (s, NH), 12.95 (s, SH). ^{13}C NMR δ: 182.0, 170.2, 163.5, 140.5, 126.5, 127.6, 128.5, 129.5, 130.9, 132.5, 134.2, 77.5. ^{31}P NMR δ: 4.98. LC-MS: *m/z* (%): 404 ($M^{+\bullet}$). *Anal.* calcd. for $C_{16}H_{13}N_4O_3PS_2$: C, 47.52; H, 3.24; N, 13.85. Found: C, 47.49; H, 3.20; N, 13.80.

3.3.2. 2-[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4b):
Yield: 85%, mp: 223-224 ºC. IR (KBr) cm^{-1}: 3316 (NH), 1657 (C=N), 1742 (P=O), 1281 (P-N). ^{1}H NMR δ: 6.94-7.82 (m, 10H), 4.27 (s, 1H), 3.86 (s, NH), 2.98 (m, 2H, CH_2), 1.10 (t, 3H, CH_3). ^{13}C NMR δ: 170.5, 168.0, 165.5, 140.5, 126.5, 129.5, 127.4, 135.5, 130.9, 128.2, 127.5, 77.5, 22.5, 13.9. ^{31}P NMR δ: 5.15. LC-MS: *m/z* (%): 400 ($M^{+\bullet}$). *Anal.* calcd. for $C_{18}H_{17}N_4O_3PS$: C, 54.00; H, 4.28; N, 13.99. Found: C, 53.49; H, 4.20; N, 13.90.

3.3.3. 2-(4-nitroanilino)-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4c):
Yield: 90%, mp: 221-222 ºC. IR (KBr) cm^{-1}: 3440(NH), 1690(P=O), 1630(C=N), 1210 (P-N). ^{1}H NMR δ: 7.12-8.24 (m, 14H), 4.48 (s, 1H), 3.60 (s, NH). ^{13}C NMR δ: 165.5, 135.0, 131.0, 127.5, 126.2, 127.0, 128.5, 127.5, 140.5, 155.5, 137.5, 118.5; 115.2; 75.5. ^{31}P NMR δ: 5.01. LC-MS: *m/z* (%): 409 ($M^{+\bullet}$). *Anal.* calcd. for $C_{20}H_{16}N_3O_5P$: C, 58.68; H, 3.94; N, 10.27. Found: C, 58.60; H, 3.91; N, 10.24.

3.3.4. 2-(4-chloroanilino)-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4d):
Yield: 86%, mp: 216-217 ºC. IR (KBr) cm^{-1}: 3392 (NH), 1746 (P=O), 1552 (C=N), 1147 (P-N). ^{1}H NMR δ: 7.46-8.03 (m, 14H), 3.76 (s, 1H), 3.62 (s, NH). ^{13}C NMR δ: 163.9, 130.8, 139.2, 141.5, 128.7, 131.1, 139.2, 129.5, 121.6, 133.8, 134.5, 123.7, 135.5,125.9, 118.9, 76.3. ^{31}P NMR δ: 6.66. LC-MS: *m/z* (%): 398 (M+1). *Anal.* calcd. for $C_{20}H_{16}ClN_2O_3P$: C, 60.24; H, 4.04; N, 7.02. Found: C, 60.20; H, 4.00; N, 6.98.

3.3.5. 5,6-diphenyl-2-(4-toluidino)-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4e):
Yield: 80%, mp: 242-243 ºC. IR (KBr) cm^{-1}: 3360 (NH), 1675 (C=N), 1690 (P=O), 1250 (P-N). ^{1}H NMR δ: 7.12-8.10 (m, 14H), 3.94 (s, 1H), 3.75 (s, NH), 2.10 (s, 3H). ^{13}C NMR δ: 140.8, 120.4, 123.9, 132.7, 127.9, 131.4, 135.4, 128.9, 124.7, 132.2, 134.8, 122.2, 135.2, 78.3. ^{31}P NMR δ: 5.08. LC-MS: *m/z* (%): 378 ($M^{+\bullet}$). *Anal.* calcd. for $C_{21}H_{19}N_2O_3P$: C, 66.66; H, 5.06; N, 7.40. Found:C, 66.54; H, 5.01; N, 7.34.

3.3.6. 2-(1H-benzo[d]imidazol-2-ylamino)-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4f):
Yield: 82%, mp: 194-195 ºC. IR (KBr) cm^{-1}: 3385 (NH), 1725 (P=O), 1680 (C=N), 1210 (P-N). ^{1}H NMR δ: 6.95-8.05 (m, 14H), 3.91 (s, 1H), 3.51 (s, NH), 4.85 (s, imadazole NH). ^{31}P NMR δ: 4.62. LC-MS: *m/z* (%): 404 ($M^{+\bullet}$). Anal. calcd. for $C_{21}H_{17}N_4O_3P$: C, 62.38; H, 4.24; N, 13.86. Found: C, 62.30; H, 4.19; N, 13.80.

3.3.7. 2-(3-chloro-4-fluroanilino)-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4g):
Yield: 84%, mp: 207-208 ºC. IR (KBr) cm^{-1}: 3400 (NH), 1710 (P=O), 1650 (C=N), 1250 (P-N). ^{1}H NMR δ: 6.80-7.82 (m, 13H), 4.20 (s, 1H), 3.52 (s, NH). ^{31}P NMRδ: 4.15. LC-MS: *m/z* (%): 416 ($M^{+\bullet}$). Anal. Calcd. for $C_{20}H_{15}N_2O_3PClF$: C, 57.64; H, 3.63; N, 6.72. Found: C, 57.60; H, 3.59; N, 6.66.

3.3.8. 2-[(4-methyl-2-pyridyl)amino]-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4h):
Yield: 82%, mp: 215-216 ºC. IR (KBr) cm^{-1}: 3350 (NH), 1730 (P=O), 1680 (C=N), 1260 (P-N). ^{1}H NMR δ: 7.19-8.17 (m, 13H), 4.39 (s, 1H), 3.61 (s, NH), 2.01 (s, 3H, CH_3). ^{31}P NMR δ: 4.80. LC-MS: *m/z* (%): 379 ($M^{+\bullet}$). *Anal.* calcd. for $C_{20}H_{18}N_3O_3P$: C, 63.32; H, 4.78; N, 11.08. Found: C, 63.29; H, 4.62; N, 11.01.

3.3.9. 5,6-diphenyl-2-(4-pyridylamino)-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4i):
Yield: 79%, mp: 203-204 ºC. IR (KBr) cm^{-1}: 3465 (NH), 1714 (C=N), 1664 (P=O), 1196 (P-N). ^{1}H NMR δ: 6.78-7.52 (m, 14H), 4.38 (s, 1H), 3.90 (s, NH). ^{31}P NMR δ: 4.83. LC-MS: *m/z* (%): 365 ($M^{+\bullet}$). *Anal.* calcd. for $C_{19}H_{16}N_3O_3P$: C, 62.47; H, 4.41; N, 11.50. Found: C, 62.30; H, 4.37; N, 11.43.

3.3.10. 2-(4-Hydroxyanilino)-5,6-diphenyl-6H-1,3,4,2λ^5-dioxazaphosphinin-2-one (4j):
Yield: 82%, mp: 261-262 ºC. IR (KBr) cm^{-1}: 3492 (NH), 1755 (C=N), 1717 (P=O), 1126 (P-N). ^{1}H NMR δ: 7.52-8.45 (m, 14H), 4.35 (s, 1H), 3.79 (s, NH), 4.90 (s, OH). ^{31}P NMR δ: 4.06. LC-MS: *m/z* (%): 380 ($M^{+\bullet}$). *Anal.* calcd. for $C_{20}H_{17}N_2O_4P$: C, 63.16; H, 4.51; N, 7.37. Found: C, 63.12; H, 4.49; N, 7.30.

3.4. Antioxidant Activity

3.4.1. DPPH Radical Scavenging Activity

The free radical scavenging activity of **4a-j** against DPPH radical was performed in accordance with Choi *et al.*[16] 85μM of DPPH was added to a medium containing different 2-substituted-5,6-diphenyl-6*H*-1,3,4,2λ^5-dioxazaphosphinin-2-ones (**4a-j**). The medium was incubated for 30 min at room temperature. The decrease in absorbance was measured at 518 nm. Ascorbic acid was used as standard reference to record maximal decrease in DPPH radical absorbance. The values are expressed in percentage of inhibition of DPPH radical absorbance with those of the standard

control values without the title compounds (**Fig. 2**) (ascorbic acid maximal inhibition was considered 100 % of inhibition).

$$DPPH\ Scavenged = \frac{(Acont - Atest)}{Acont} \times 100$$

Where Acont is the absorbance of the control reaction and Atest is the absorbance in the presence of the sample.

In the case of **4a-j**, nitro substituted compound **4c** showed the highest DPPH radical scavenging activity with IC_{50} at 49.3 µg/mL when compared with others. The remaining compounds exhibited DPPH radical scavenging activity in the following order: **4j** (IC_{50} 52.7 µg/mL), **4g** (IC_{50} 59.8 µg/mL), **4a** (IC_{50} 60.1 µg/mL), **4b** (IC_{50} 62.1 µg/mL), **4h** (IC_{50} 62.2 µg/mL), **4e** (IC_{50} 63.4 µg/mL), **4d** (IC_{50} 66.5 µg/mL), **4f** (IC_{50} 68.2 µg/mL), **4i** (IC_{50} 70.8 µg/mL) and when compared with ascorbic acid (IC_{50} 50.0 µg/mL).

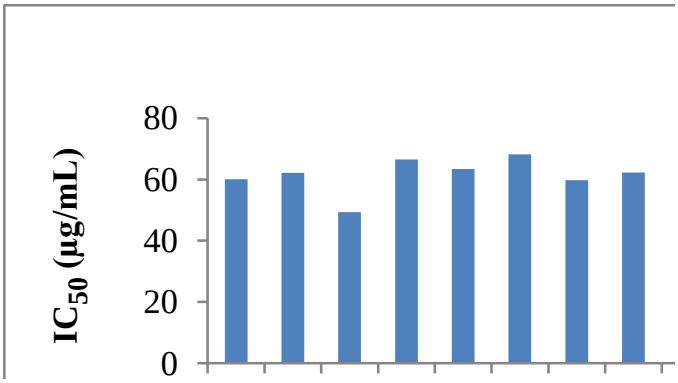

Figure 2. DPPH Radical Scavenging Activity of 4a-j

3.4.2. NO Scavenging Activity

The scavenging activity of **4a-j** against Nitric Oxide radical was performed in accordance with Shirwaiker *et al.*[17] Sodium nitropruside (5 µM) in phosphate buffer pH 7.4was incubated with 100 µM concentration of test compounds dissolved in a suitable solvent (dioxane/ methanol) and tubes were incubated at 25 ^{o}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*-naphthyl ethylene diamine dihydro chloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthyl ethylene diamine dihydrochloride was read at λ 546 nm.

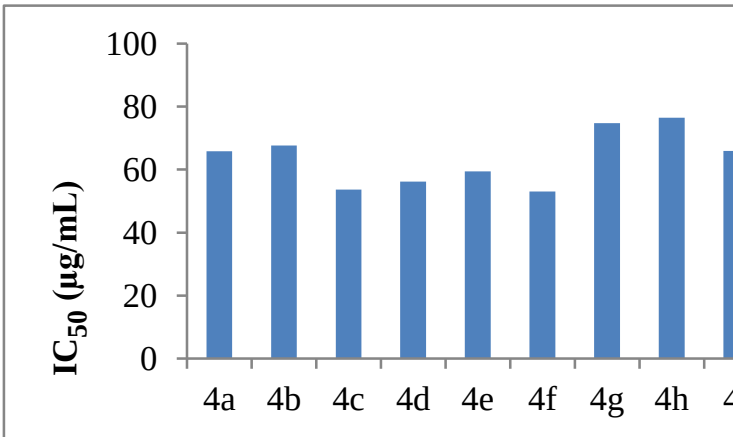

Figure 3. NO Scavenging Activity of 4a-j

In the case of 2-substituted-5,6-diphenyl-6*H*-1,3,4,2λ^5-dioxazaphosphinin-2-ones **4a-j** (**Fig. 3**) derivatives, **4f** showed the highest NO scavenging with IC_{50} of 53.0 µg/mL when compared with other compounds. The remaining compounds NO scavenging activity in the following order: **4c** (IC_{50} 53.7 µg/mL), **4d** (IC_{50} 56.2 µg/mL), **4e** (IC_{50} 59.4 µg/mL), **4a** (IC_{50} 65.8 µg/mL), **4i** (IC_{50} 65.9 µg/mL), **4b** (IC_{50} 67.7 µg/mL), **4g** (IC_{50} 74.8 µg/mL), **4h** (IC_{50} 76.5 µg/mL), **4j** (IC_{50} 81.9 µg/mL) and when compared with ascorbic acid (IC_{50} 64.9 µg/mL).

3.4.3. Reducing power assay

The reducing power of **4a-j** was determined according to the method of Oyaizu *et al.*[18] The compounds having 50-100 µM were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and incubated at 50°C for 20 min. To this mixture 2.5 mL of 10% trichloroacetic acid (TCA) was added and the mixture was centrifuged at 3000 rpm for 20 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% Ferric chloride and the UV absorbance was measured at 700 nm using a spectrophotometer. Increase of absorbance of the reaction mixture indicates higher reducing power. Mean values from three independent samples were calculated for each compound and standard deviations were less than 5 %.

In the case of 2-substituted-5,6-diphenyl-6*H*-1,3,4,2λ^5-dioxazaphosphinin-2-ones **4a-j** (**Fig. 4**) derivatives **4c** showed the highest reducing power with IC_{50} of 1.75 µg/mL when compared with other compounds. The remaining compounds exhibited reducing power activity in the following order: **4f** (IC_{50} 1.86 µg/mL), **4a** (IC_{50} 2.09 µg/mL), **4e** (IC_{50} 2.21 µg/mL), **4d** (IC_{50} 2.35 µg/mL), **4b** (IC_{50} 2.63 µg/mL), **4i** (IC_{50} 2.81 µg/mL), **4g** (IC_{50} 3.03 µg/mL), **4j** (IC_{50} 3.12 µg/mL), **4h** (IC_{50} 3**.38** µg/mL) and when compared with ascorbic acid (IC_{50} 2.42 µg/mL).

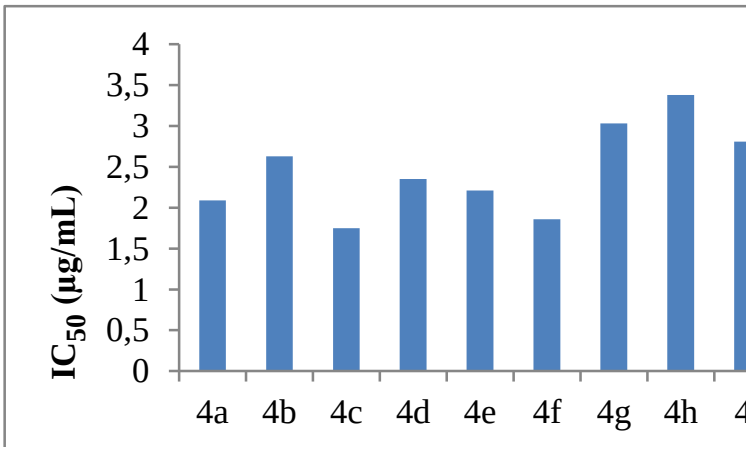

Figure 4. Reducing Power Assay of 4a-j

4. Conclusions

In conclusion, dioxazaphosphinin-2-ones were synthesized in good to moderate yields. All the title compounds **4a-j** were tested for their antioxidant activity by three methods namely DPPH, NO and Reducing power assay methods and they showed the activity in high to moderate. The main advantages of the present method are operational simplicity, mild, eco-friendly reaction conditions, short reaction times, easy reaction work-up procedure and good product yields.

Acknowledgements

The authors express their grateful thanks to Prof. C.D. Reddy, Department of Chemistry, Sri Venkateswara University, Tirupati, for his helpful discussions and also the University Grant Commission (UGC) for providing financial assistance (F. No.: 42-281/2013 (SR), Dated: 12-03-2013).

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