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Synthesis, Antibacterial and Antioxidant Properties of Newer 1,2-benzoxaphosphol-2-ones

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Abstract

A new series of 5-chloro-3-(4-substituted anilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-ones **4a-j** have been prepared via Mannich type reaction by reacting 5-chlorosalicyaldehyde **1** with phenyl dichlorphosphine **2** and aromatic amines **3a-j** in anhydrous benzene under reflux condition. Their chemical structures were characterized by using ^{1}H, ^{13}C, ^{31}P and mass spectral studies. All the title compounds were screened for antioxidant properties by radical scavenging methods such as 1,1-dipheyl-2-picryl hydrazyl (DPPH), Reducing power and H_2O_2 scavenging methods. They exhibited potent *in vitro* antioxidant activity dose dependently. Their bioassay showed that they possess significant antibacterial activity.

Key words: Mannich-type reaction, spectral analysis, antioxidant, anti-bacterial activity.

Introduction

The chemistry of organophosphorus heterocycles has always attracted much attention because of their unique potential biological properties such as systemic pesticides, antifungal/antibacterial/antileukemic/antiparasitic/antiviral/antiinflammatory/antitumoral/antihypertensive, antioxidant[1-7] and therefore a large number of related compounds have been synthesized in the past two decades. On the other hand, phosphorus heterocyclic compounds received much attention of chemists due to their pharmaceutical importance [8,9] and extensive application in organic synthesis [10]. This background prompted us synthesis of new benzoxaphosphol-2-ones as continuation of our research in phosphorus heterocycles [11]. Syntheses of 5-chloro-3-(4-substituted anilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one derivatives are presently reported.

Antioxidants are predominately studied as first line therapy that protects organisms from deleterious effects induced by oxidative stress during metabolism. Search for active components that prevent or reduce the impact of oxidative stress on cells is a contemporary field [12]. Biological reduction of molecular oxygen (O_2) generates products collectively termed reactive oxygen species (ROS). By accepting a single electron, O_2 is transformed into the superoxide radical anion. O_2^-, which displays a key role in biological systems. Superoxide radicals are generated under natural conditions during mitochondrial respiration by UV-B radiation and in phagocytosis of cells engaged in immune response. The superoxide radical anion is the substrate for the most reactive form of ROS generated in the Haber-weiss and Fentons reaction [13]. Increased free radical generation, especially superoxide anion in leukemia patients and increased antioxidant defense enzymes, which is an adaptive protective response are indicative of mild oxidative stress. ROS exhibit a wide spectrum of pathogenic properties. They are capable of oxidizing biomolecules and cause cell death and consequently tissue damage. 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 pathological abnormalities such as inflammation, diabetes, genotoxicity and cancer [14]. In the present investigation, radical scavenging and antioxidative capacity for the newly synthesized compounds are evaluated using three anti-oxidant methodologies.

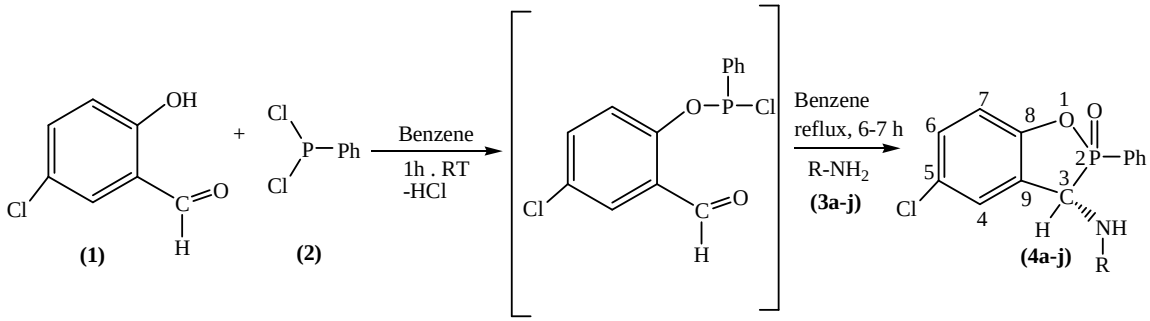
Results and Discussion

Herein, we report a convenient and an efficient synthesis of 5-chloro-3-(4-substituted anilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one derivatives **4a-j** via a facile straight forward method. The Mannich-type reaction of trivalent phopshines has proved as a facile method for the preparation of new phosphorus heterocyclic compounds [15]. As shown in **Scheme-1**, 5-chlorosalicyaldehyde **1** was allowed to react with phenyl dichlorophosphine **2** and various substituted aromatic primary amines **3a-j** in refluxing anhydrous benzene for 6-7 h to obtain 72-90% yields of the title compounds **4a-j**.

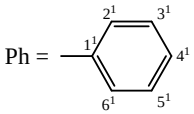
Characteristic IR absorptions were observed in the regions 1242-1265 cm^{-1} for P=O and 3317-3364 cm^{-1} for –NH stretching frequencies for **4a-j** [16].The aromatic hydrogens resonated as multiplets at δ 6.51-7.91 in their ^{1}H-NMR spectra. The P-N-H hydrogen chemical shift appeared as a singlet [17] at δ 4.01- 4.12. The P-C-H hydrogen signal appeared as a doublet at δ 5.02-5.12 (d, *J*= 7.6-8.4 Hz). Theoretically, formation of two stereoisomers for the product **4** is possible in this reaction because of the presence of two different substituents in the rigid oxaphosphole ring at C_3 and Phosphorus atom. The ^{13}C NMR chemical shifts for **4a-j** appeared in their expected regions [16,17]. The ^{31}P NMR chemical shifts were observed at δ 30.26- 33.19 [18].

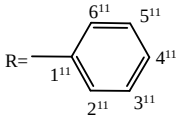
The radical scavenging capacity **4a-j** was evaluated by methods such as 1,1-diphenyl-2-picryl hydarzyl(DPPH) **Fig.1**, Reducing power **Fig.2** and H_2O_2 scavenging techniques **Fig.3**. **4c**, **4g** and **4i** displayed appreciable antioxidant activity. **4i** showed the highest activity because, both fluorine and –NO_2 substituents which affect the electron and hydrogen donating capacities appears to be useful in inducing antioxidant activity. Since fluorine shows highly negative inductive effect and –NO_2 is highly withdrawing moiety thereby, electron density around P=O moiety decreases and increases affinity towards oxygen derived free radicals and mobilizes ROS to be scavenged out of living system. Besides, due to presence of a chlorine atom on aryl ring in

4a-j can strongly polarize that parent molecule and seems to have profound effect on biological properties of title compounds. This points to the fact that electron withdrawing substituent in **4a-j** appears to prevent to some extent oxidative metabolic pathways in the living system.



Entry	R	Entry	R
3a & 4a	C_6H_5	3f & 4f	$C_6H_4NO_2$(3')
3b & 4b	C_5H_4N	3g & 4g	C_6H_3Cl(3') Cl (4')
3c & 4c	C_6H_4Cl(4')	3h & 4h	C_6H_3F(4') Cl (3')
3d & 4d	C_6H_4F(4')	3i & 4i	C_6H_3F(4') NO_2 (3')
3e & 4e	$C_6H_4CH_3$(4')	3j & 4j	C_7H_4SN





Synthetic Scheme -1 for 4a-j

Biological Activity

Antioxidant Activity. 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical Scavenging Activity. The hydrogen atom or electron donation abilities of the corresponding synthesized compounds **4a-j** were measured from the bleaching of the purple-colored methanol solution of 2, 2'-diphenly-1-picrylhydrazyl (DPPH) is presented in **Fig. 1.** This spectrophotometric assay uses the stable radical DPPH as a reagent [19]. 1ml of various concentrations of the synthesized compounds **4a-j** (25, 50, 75, 100 and 250µg/ml) in methanol were added to 4ml of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm.

The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{blank}] \times 100.$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} is the absorbance of the test compound and A_{blank} is only methanol.

Tests were carried out in triplicate. In the case of 1,2-benzoxaphosphol-2-one derivatives **4a-j**, **4i** showed the highest DPPH scavenging activity with IC_{50} of 46.25 µg/ml when compared with other compounds. The remaining compounds exhibited DPPH radical scavenging activity in the following order: **4g** (IC_{50} 47.25 µg/ml), **4c**(IC_{50} 53.59 µg/ml), **4e**(IC_{50} 107.50 µg/ml), **4h** (IC_{50} 124.56 µg/ml), **4d** (IC_{50} 126.24 µg/ml), **4a** (IC_{50} 303.30 µg/ml), **4f** (IC_{50} 657.22 µg/ml), **4j** (IC_{50} 713.77 µg/ml), **4b** (IC_{50} 734.70 µg/ml) and when compared with BHT (IC_{50} 56.09 µg/ml).

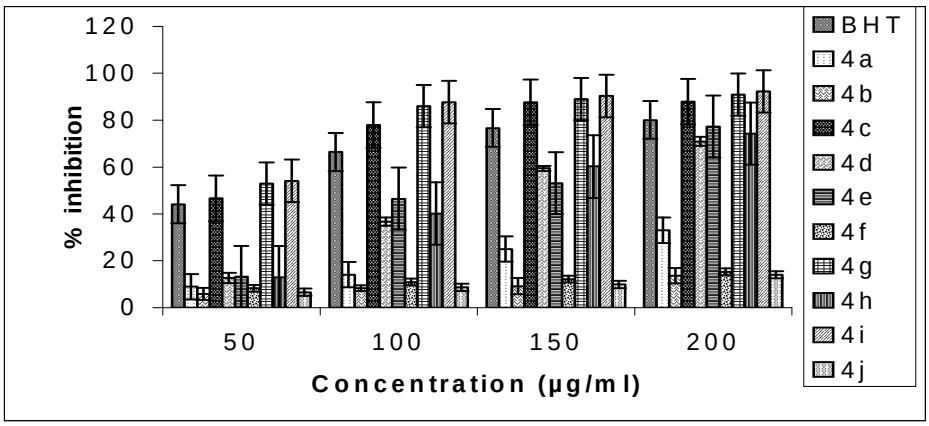


Fig. 1. DPPH Radical scavenging activity of compounds 4a-j

Reducing Power

The reducing power was determined according to the method of Oyaizu [20]. Different concentrations of synthesized compounds **4a-j** (25, 50, 250 and 500µg/ml) prepared in methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5ml, 1%).

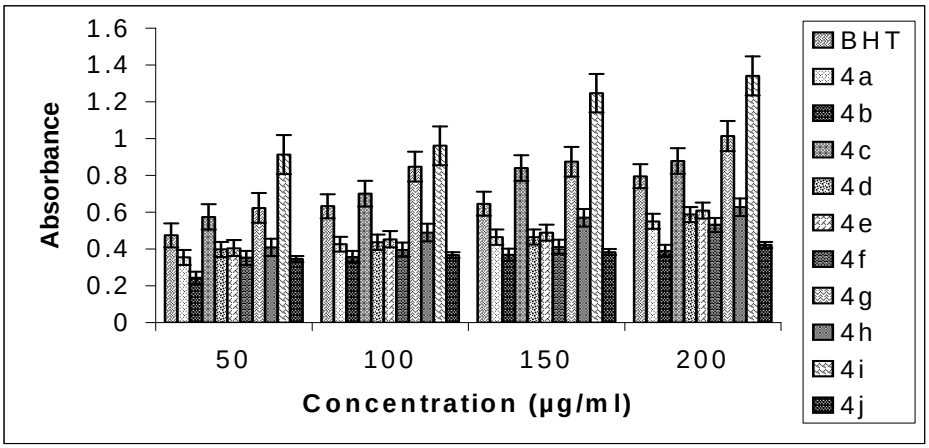


Fig. 2. Reducing power activity of compounds 4a-j

The mixture was incubated at 50°C for 20 min and 2.5ml of trichloroaceticacid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the

absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. BHT was used as a standard. In the case of 1,2-benzoxaphosphol-2-one derivatives **4a-j**, the absorbance values are displayed in **Fig.2** because the compounds show reducing activity. **4i** showed the highest reducing power activity when compared with other compounds. The remaining compounds exhibited reducing power activity in the following order: **4g > 4c 4e> 4h > 4d > 4a> 4f > 4b > 4j** and when compared with BHT.

H_2O_2 scavenging activity

The H_2O_2 scavenging ability of the synthesized compounds **4a-j** were determined according to the method of Ruch *et al* [21]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 10, 25, 50, 75 &100 µg/ml concentrations of the synthesized compounds **4a-j** in 3.4 ml phosphate buffer were added to a H_2O_2 solution (0.6ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm.

The H_2O_2 scavenging activity (%) was calculated by using the following equation:

H_2O_2 scavenging activity (%) = $[(A_{control} - A_{sample})/A_{blank}] \times 100$.

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Tests were carried out in triplicate. In the case of 1,2-benzoxaphosphol-2-one derivatives **4a-j**, **4i** showed the highest H_2O_2 scavenging activity with IC_{50} of 28.38 µg/ml when compared with other compounds. The remaining compounds exhibited H_2O_2 scavenging activity in the following order: **4g** (IC_{50} 29.44 µg/ml) **4c**(IC_{50} 29.89 µg/ml) **4e**(IC_{50} 37.70 µg/ml) **4h** (IC_{50} 40.65 µg/ml) **4d** (IC_{50} 42.95 µg/ml) **4a** (IC_{50} 46.90 µg/ml) **4f** (IC_{50} 48.26 µg/ml) **4b** (IC_{50} 53.99 µg/ml) **4j** (IC_{50} 101.6 µg/ml) and when compared with BHT (IC_{50} 36.10 µg/ml).

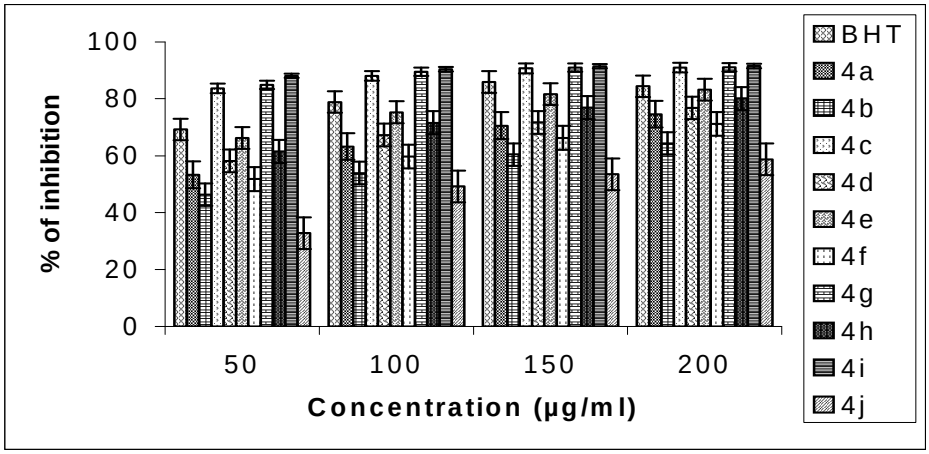


Fig. 3. H_2O_2 scavenging activity of compounds 4a-j

Bioassay

Agar well bioassay was employed for testing antibacterial and antifungal activity of **4a-j (Table-1)**. Diluted inoculum 0.1 ml (10^5 CFU / ml) of bacteria was spread on nutrient agar and fungi on Potato Dextrose Agar plates (PDA). Wells of 8 mm were punched into the agar medium and

filled with the title compounds at the concentration of 25 and 50 µg in each well. The plates were incubated for 24 h at 37°C for test bacteria and the fungi plates were incubated for 72 h at 28°C. The antimicrobial activity was evaluated by measuring the zone of inhibition against test organisms. Chloramphenicol and Ketoconazole were used as commercial standards. Controls were maintained with dimethyl sulphoxide (DMSO) [22].

Determination of MIC

Minimum Inhibitory Concentration (MIC) was determined for the compounds **4a-j (Table-2)** that showed total growth inhibition using the protocol described below. The minimum concentration, at which there was no visually detectable bacterial growth, was taken as MIC. The compound concentration of 10 µg to 50 µg/ml in steps of 10 µg/ml was evaluated. Specifically 0.1 ml of standardized inoculum (1-2 x 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. The method followed for antifungal bioassay is similar to that followed for antibacterial assay wherein the medium is PDA and incubation temperature is 28°C for 72 h [23].

Table-1: Diameter of Zone of Inhibition (in mm) of 1,2-benzoxaphosphol-2-ones (µg/ml)

Entry	*Staphylococcus aureus*		*Bacillus subtilis*		*Escherichia coli*		*Klebsiella pneumoniae*		*Salmonella typhimurium*		*Candida albicans*		*Aspergillus niger*	
	25	50	25	50	25	50	25	50	25	50	25	50	25	50
4a	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4b	10	12	10	12	-	12	-	12	-	12	-	10	-	10
4c	-	12	-	12	-	12	-	12	-	12	-	10	-	10
4d	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4e	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4f	10	12	10	12	-	10	-	10	-	10	-	10	-	10
4g	10	12	10	12	-	12	-	12	-	12	-	10	-	10
4h	12	15	12	15	12	15	12	15	12	15	10	12	10	12
4i	-	10	-	10	-	10	-	10	-	10	-	-	-	-
4j	-	10	-	10	-	10	-	10	-	10	-	-	-	-
Chloramphenicol (5)	-	25	26		22		30		-	22	-		-	
Ketoconazole (50)	-	-	-		-		-		-	-	-		-	
			-		-		-		-		16		18	

Each well contains 25 and 50 µg of compounds. Ch = Chloramphenicol 5 µg / well, Ketoconazole 50 µg/well

Table 2: Minimium Inhibition Concentration of 1,2-benzoxaphosphol-2-ones (µg/ml)

Entry	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhimurium*	*Candida albicans*	*Aspergillus niger*
4a	0.16	0.17	0.22	0.21	0.20	0.29	0.27
4b	0.28	0.31	0.3	0.31	0.32	0.33	0.34
4c	0.29	0.30	0.33	0.32	0.33	0.34	0.32
4d	0.30	0.32	0.32	0.29	0.30	0.40	0.38
4e	0.32	0.31	0.36	0.33	0.31	0.34	0.37
4f	0.41	0.41	0.50	0.50	0.47	0.40	0.38
4g	0.46	0.48	0.44	0.44	0.39	0.37	0.38
4h	0.42	0.44	0.40	0.47	0.42	0.41	0.40
4i	0.46	0.44	0.50	0.50	0.47	0.46	0.45
4j	0.45	0.46	0.40	0.40	0.44	-	-

Experimental

General

The melting points were determined in an 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 Brucker -AMX spectrometer operating at 500 MHz for ^{1}H and 125.0 MHz for ^{13}C. ^{31}P-NMR spectra were recorded on a Brucker-AMX -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). APCI mass spectra were recorded on LCMS-2010A Shimadzu instrument. 5-Chlorosalicyaldehyde **1** and phenyl dichlorophosphine **2** and various aromatic amines **3a-j** were procured from Sigma-Aldrich Chemical Company, Milwaukee, U.S.A. and were used without further purification.

General Procedure for synthesis of 4a-j

To a stirred solution of 5-chlorosalicyaldehyde **1** (0.78 g, 5mmol), dichloro phenyl phosphine **2**, (0.67 g, 5mmol) in anhydrous benzene (15 ml) was added dropwise. Stirring was continued at room temperature for 1h. The aniline **3a** (0.45 g, 5mmol) in anhydrous benzene (15 ml) was added dropwise. Stirring was continued at room temperature for 0.5 h, after which the mixture was heated under reflux for 6-7 h. After cooling, a white precipitate was formed, collected by filtration and it was purified by washing with water followed by recrystallization from 2-propanol.

3-Anilino-5-chloro-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4a:**

White solid: Yield was found to be 77%, mp 202-204 ºC. IR (KBr) cm^{-1}:1245 (P=O), 3342(-NH). ^{1}H-NMR (DMSO-d_6): δ 6.51-7.72 (m, 13H, Ar-H), 4.01 (s, 1H, NH), 5.06 (d, J = 8.2 Hz, 1H, P-CH). ^{13}C-NMR (DMSO-d_6): δ 51.21(d, J=107.0 Hz)(C-3), 128.27 (C-4), 126.41 (C-5), 128.54 (C-6), 116.88 (C-7), 154.92 (C-8), 131.90 (C-9), 144.28 (C-1'), 115.53 (C-4'), 114.24 (C-2' &C-6'), 129.08 (C-3' &C-5'), 130.99 (C-1"),132.31 (C-4"), 133.94 (C-2"& C-6"), 128.58 (C-3"& C-5"). ^{31}P-NMR (DMSO-d_6): δ 30.51. LCMS: m/z (%) 355, 357 (M+2). *Anal*. Calcd. for $C_{19}H_{15}ClNO_2P$: C, 64.15; H, 4.25; N, 3.94. Found C, 64.06; H, 4.20; N, 3.89.

5-Chloro-2-phenyl-3-(4-pyridylamino)-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4b:**

White solid: Yield was found to be 86%, mp 190-192 ºC. IR (KBr) cm^{-1}:1265 (P=O), 3317(NH). ^{1}H-NMR (DMSO-d_6): δ 6.54-7.74 (m, 12H, Ar-H), 4.12 (s, 1H, NH), 5.12 (d, *J*=8.2 Hz, 1H, P-CH). ^{31}P-NMR (DMSO-d_6): δ 33.19. *Anal*. Calcd. for $C_{18}H_{14}ClN_2O_2P$: C, 60.60; H, 3.96; N, 7.85. Found C, 60.51; H, 3.91; N, 7.81.

5-Chloro-3-(4-chloroanilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4c:**
White solid: Yield was found to be 84%, mp 172-174ºC. IR (KBr) cm^{-1}:1246 (P=O), 3343(NH). ^{1}H-NMR (DMSO-d_6): δ 6.67-7.85 (m, 12H, Ar-H), 4.04 (s, 1H, NH), 5.02 (d, *J* = 8.1 Hz, 1H, P-CH). ^{31}P-NMR (DMSO-d_6): δ 31.21. *Anal*. Calcd. for $C_{19}H_{14}Cl_2NO_2P$: C, 58.48; H, 3.62; N, 3.59. Found C, 58.39; H, 3.57; N, 3.54.

5-Chloro-3-(4-fluoroanilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4d:**
White solid: Yield was found to be 90%, mp 184-186ºC. IR (KBr) cm^{-1}:1242 (P=O), 3352(NH). ^{1}H-NMR (DMSO-d_6): δ 6.68-7.91 (m, 12H, Ar-H), 4.06 (s, 1H, NH), 5.06 (d, *J* = 7.6 Hz, 1H, P-CH). ^{13}C-NMR (DMSO-d_6): δ 50.28(d, *J*=107.0 Hz, (C-3), 128.25 (C-4), 126.40 (C-5), 128.52 (C-6), 116.86 (C-7), 154.90 (C-8), 131.88 (C-9), 143.20 (C-1'), 148.30 (C-4'), 113.24 (C-2' &C-6'), 128.08 (C-3' &C-5'), 130.97 (C-1"),132.28 (C-4"), 132.93 (C-2"& C-6"), 128.56 (C-3"& C-5"). ^{31}P-NMR (DMSO-d_6): δ 30.26. LCMS: m/z (%) 373, 375(M+2). *Anal*. Calcd. for $C_{19}H_{14}ClFNO_2P$: C, 61.06; H, 3.78; N, 3.75. Found C, 60.97; H, 3.73; N, 3.70.

5-Chloro-3-(4-methylanilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4e:**
White solid: Yield was found to be 78%, mp 166-168ºC. IR (KBr) cm^{-1}:1247 (P=O), 3346 (NH). ^{1}H-NMR (DMSO-d_6): δ 6.61-7.75 (m, 12H, Ar-H), 4.08 (s, 1H, NH), 5.09 (d, *J* = 8.1 Hz, 1H, P-CH). ^{31}P-NMR (DMSO-d_6): δ 32.19. *Anal*. Calcd. for $C_{20}H_{17}ClNO_2P$: C, 64.96; H, 4.63; N, 3.79. Found C, 64.88; H, 4.58; N, 3.74.

5-Chloro-3-(3-nitroanilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4f:**
White solid: Yield was found to be 75%, mp 178-180ºC. IR (KBr) cm^{-1}:1248 (P=O), 3348(NH). ^{1}H-NMR (DMSO-d_6): δ 6.62-7.86 (m, 12H, Ar-H), 4.08 (s, 1H, NH), 5.12 (d, *J* = 7.8 Hz, 1H, P-CH). ^{13}C-NMR (DMSO-d_6): δ 51.18(d, *J*=108.0 Hz)(C-3), 129.26 (C-4), 127.42 (C-5), 128.52 (C-6), 117.86 (C-7), 154.92 (C-8), 131.78 (C-9), 146.52 (C-1'), 110.42 (C-2'), 149.52 (C-3'), 113.32 (C-4'), 131.52(C-5'), 117.62(C-6'), 130.96 (C-1"),132.32 (C-4"), 132.94(C-2"& C-6"), 127.54 (C-3"& C-5"). ^{31}P-NMR (DMSO-d_6): δ 31.26. LCMS: m/z (%) 400, 402 (M+2) *Anal*. Calcd. for $C_{19}H_{14}ClN_2O_4P$: C, 56.94; H, 3.52; N, 6.99. Found C, 56.85; H, 3.47; N, 6.94.

5-Chloro-3-(3,4,dichloroanilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4g:**
White solid: Yield was found to be 76%, mp 172-174ºC. IR (KBr) cm^{-1}:1262 (P=O),3362 (NH). ^{1}H-NMR (DMSO-d_6): δ 6.64-7.84 (m, 11H, Ar-H), 4.11 (s, 1H, NH), 5.08 (d, *J* = 8.4 Hz, 1H, P-CH). ^{31}P-NMR (DMSO-d_6): δ 30.46. *Anal*. Calcd. for $C_{19}H_{13}Cl_3NO_2P$: C, 53.74; H, 3.09; N, 3.30. Found C, 53.65; H, 3.04; N, 3.25.

5-Chloro-3-(3-chloro 4-fluoro anilino)-2-phenyl-2,3-dihydro-1,2λ^{5}benzoxaphosphol-2-one **4h:**
White solid: Yield was found to be 85%, mp 167-169ºC. IR (KBr)cm^1:1252(P=O), 3353 (NH). ^{1}H-NMR (DMSO-d_6): δ 6.64-7.89 (m, 11H, Ar-H), 4.08 (s, 1H, NH), 5.12 (d, *J* = 8.2 Hz, 1H, P-CH). ^{13}C-NMR (DMSO-d_6): δ 51.16(d, *J*=107.0 Hz)(C-3), 129.28 (C-4), 127.46 (C-5), 128.56 (C-

6), 118.86 (C-7), 152.90 (C-8), 130.88 (C-9), 146.54 (C-1'), 114.52 (C-2'), 122.52 (C-3'),152.22 (C-4'), 119.24 (C-5'),112.26 (C-6'), 129.94 (C-1"),132.28 (C-4"), 133.82 (C-2"& C-6"), 128.56 (C-3"& C-5"). ^{31}P-NMR (DMSO-d_6): δ 31.58. LCMS: m/z (%) 407, 409(M+2). *Anal*. Calcd. for $C_{19}H_{13}Cl_2FNO_2P$: C, 55.91; H, 3.21; N, 3.43. Found C, 55.82; H, 3.16; N, 3.38

5-Chloro-3-(4-fluoro 3-nitro anilino)-2-phenyl-2,3-dihydro-1,2λ^5-benzoxaphosphol-2-one **4i:**
White solid: Yield was found to be 82%, mp 168-170ºC. IR (KBr) cm^{-1}:1247 (P=O), 3364 (NH). ^{1}H-NMR (DMSO-d_6): δ 6.72-7.89 (m, 11H, Ar-H), 4.08 (s, 1H, NH), 5.06 (d, *J* = 7.6 Hz, 1H, P-CH). ^{13}C-NMR (DMSO-d_6): δ 48.28(d, *J*=107.0 Hz)(C-3), 129.22 (C-4), 126.38 (C-5), 128.48 (C-6), 116.84 (C-7), 154.88 (C-8), 131.86 (C-9), 142.24 (C-1'), 112.34 (C-2'), 138.62 (C-3'), 149.43 (C-4'), 118.24(C-5'), 123.24(C-6'), 143.97 (C-1"),131.27 (C-4"), 132.94 (C-2"& C-6"), 126.54 (C-3"& C-5"). ^{31}P-NMR (DMSO-d_6): δ 31.48. *Anal*. Calcd. for $C_{19}H_{13}ClFN_2O_4P$: C, 54.50; H, 3.13; N, 6.69. Found C, 54.41; H, 3.08; N, 6.65.

5-Chloro-3-(2,3-dihydro-1,3-benzothiazol-2-ylamino)-2-phenyl-2,3-dihydro-1,2λ^5- benzoxapho-sphol-2-one **4j:**
White solid: Yield was found to be 72%, mp 210-212ºC. IR (KBr) cm^{-1}:1246 (P=O),3358 (-NH). ^{1}H-NMR (DMSO-d_6): δ 6.56-7.85 (m, 12H, Ar-H), 4.10 (s, 1H, NH), 5.09 (d, *J* = 7.6 Hz, 1H, P-CH). ^{31}P-NMR (DMSO-d_6): δ 31.32. *Anal*. Calcd. for $C_{20}H_{14}ClN_2O_2PS$: C, 58.19; H, 3.42; N, 6.79. Found C, 58.11; H, 3.37; N, 6.74

Conclusion

In conclusion, synthesis of a series of novel benzoxaphosphol-2-ones **4a-j** in high yields was accomplished. Some of them were found to possess potent antioxidant activity when compared to that of the standard BHT and significant antimicrobial activity. These results encourage further *in vivo* studies and explore their possible therapeutic applications

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