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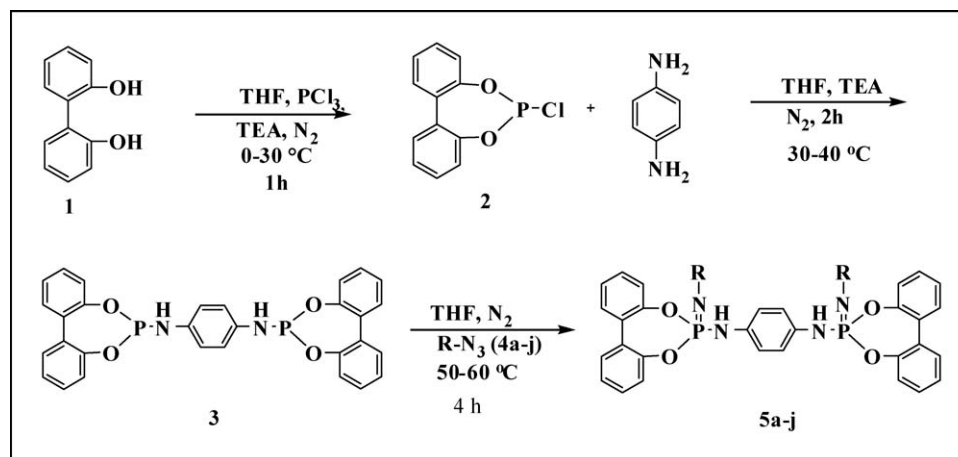
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A new class of novel benzene-1,4-diamine-bis-dioxaphosphepine-6 λ^5 iminophosphoranes (**5a-j**) were synthesized by the reaction of 6-chlorodibenzo[*d,f*][1,3,2]dioxaphosphepine (**2**) with 1,4-diaminobenzene to form bis-dibenzo[*d,f*][1,3,2]dioxaphosphepin-6-yl-benzene-diamine (**3**). Its subsequent reaction with different alkyl/aryl azides (**4a-j**) in tetrahydrofuran at 50–60°C under inert atmosphere yielded title compounds. Their structures were established by elemental analysis, IR, ^1H , ^{13}C , ^{31}P NMR, and mass spectral studies. All the title compounds were screened for antioxidant properties and found to exhibit potent *in vitro* antioxidant and antimicrobial activity

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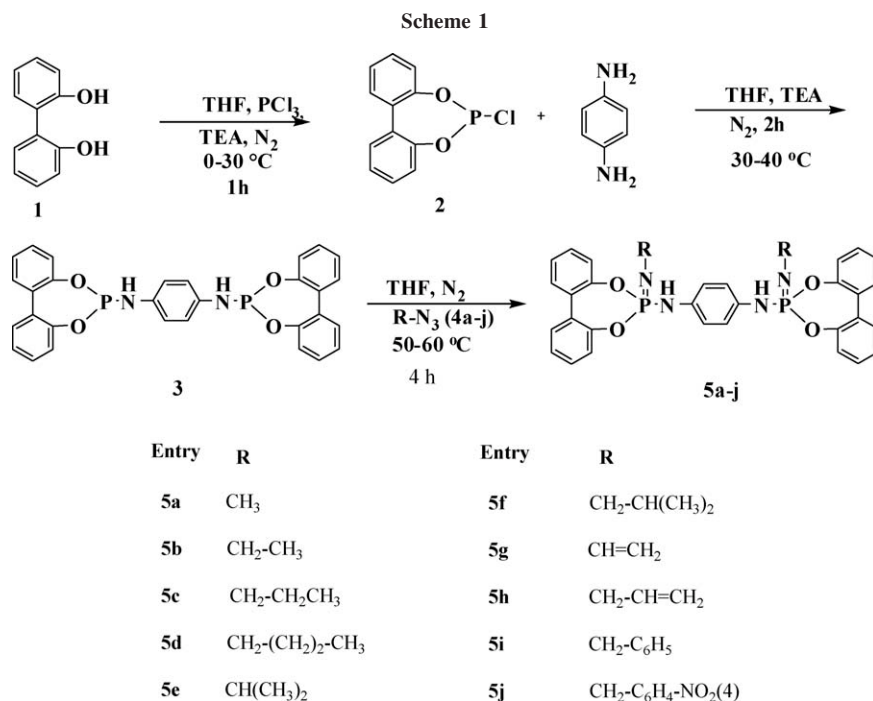
INTRODUCTION

In recent years, the detailed mechanism of antioxidant action of organophosphorus compounds and their relationships between chemical structure and antioxidant activity have been comprehensively studied, in spite of their great practical importance. Depending on their structure and scavenging activity, phosphites and phosphonates may act as both primary and secondary antioxidants [1,2]. Reactive oxygen species (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 inflammatory process in rheumatoid arthritis (RA) and contribute to the destruction of cartilage and bone [3,4]. The most important ROS implicated in inflammatory tissue injury are superoxide radical ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^\bullet), and hypochlorous acid (HOCl^\bullet). In the inflamed joint, these ROS can be produced by macrophages, neutro-

phils, and chondrocytes [5]. The inflamed rheumatoid joint also undergoes a hypoxia–reperfusion cycle, which results in ROS generation [6]. Antioxidants may have a therapeutic role in RA by suppressing the inflammation. As our synthesized compounds are contrast in the structure to bisphosphonates, the aim of this study was to investigate the *in vitro* antioxidant profile of different bis-iminophosphoranes. To the best of our knowledge the antioxidant profile of different bis-iminophosphoranes has not yet been systematically studied.

RESULTS AND DISCUSSION

Cyclization of 2,2'-dihydroxybiphenyl (**1**) with phosphorus trichloride at 0°C under dry and inert conditions in the presence of triethylamine (TEA) in tetrahydrofuran (THF) afforded the corresponding 6-chlorodibenzo[*d,f*][1,3,2]dioxaphosphepine (**2**). Reaction of **2** with 1,4-diaminobenzene led to bis dibenzo[*d,f*][1,3,2]dioxaphosphepin-6-yl-1,4-benzenediamine (**3**). Further reac-



tion of **3** with different organic azides **4a-j** in dry THF at 50–60°C led to **5a-j** in high yields (Scheme 1). The reactions were monitored by thin layer chromatography (TLC). The chemical structures of **5a-j** were confirmed by elemental analysis and spectral data (IR, ¹H, ¹³C, ³¹P NMR, and mass spectra).

Characteristic IR absorptions were observed for C–N, P=N, and NH in the regions 1010–1081, 1206–1265, and 3280–3410 cm⁻¹, respectively [7]. The aromatic hydrogens resonated as multiplets at δ 6.45–7.99, the P–NH proton chemical shift appeared as a singlet at δ 3.75–5.20. The chemical shifts of other aliphatic hydrogens and carbon-13 **5a-j** appeared in the expected region [8]. ³¹P NMR chemical shifts were observed in the region δ 4.25–9.20 [7]. LCMS of **5a**, **5b**, **5e**, **5g**, **5i**, and **5j** gave molecular ion peaks and diagnostic daughter ions at their expected m/z values.

The radical scavenging capacity of **5a-j** was evaluated by using methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide scavenging activity. **5j** showed appreciable antioxidant activity. Because of –NO₂ substituents which affect the electron and hydrogen donating capacities, appears to be useful in inducing antioxidant activity. As –NO₂ is highly withdrawing moiety, thereby electron density around phosphonate moiety decrease and increases affinity toward oxygen derived free radicals and mobilizes ROS to be scavenged out of living system.

EXPERIMENTAL

All melting points were determined in open capillary tubes on Mel-Temp apparatus and are uncorrected. Microanalyses

were performed at the Central Drug Research Institute, Lucknow, India. Infrared spectra (ν_{\max} in cm⁻¹) were recorded as KBr pellets on a Perkin-Elmer 283 double beam spectrophotometer. ¹H, ¹³C, and ³¹P NMR spectra were recorded on AMX 400 MHz spectrophotometer operating at 400 MHz for ¹H NMR, 100 MHz for ¹³C NMR, and 161.9 MHz for ³¹P NMR, using DMSO-*d*₆ as solvent. The ¹H and ¹³C NMR chemical shifts were referenced to Tetra Methyl Silane (TMS) and ³¹P NMR chemical shifts to 85% H₃PO₄.

Typical experimental procedure.

Preparation of alkyl azides. In a dry 100-mL round-bottomed flask fitted with dropping funnel, calcium chloride guard tube, sodium azide (0.64 g, 0.01 mole), and 10 mL of dry THF were placed and stirred. Alkyl/aryl bromide (0.01 mole) in 10 mL of dry THF was added to it at room temperature. Temperature of the reaction mixture was raised to 40–45°C and stirred for 4 h. After cooling to room temperature, it was filtered to remove sodium bromide. The filtrate containing alkyl/aryl azide was used for the next step reaction.

N1,N4 Bis[6-(alkyl/arylimino)-6 λ^5 -dibenzo[d,f][1,3,2]dioxaphosphepine-6yl]benzene-1,4-diamine 5a-j. A solution of slight excess of phosphorus trichloride (0.43 g, 0.005 mole) in dry THF (25 mL) under nitrogen atmosphere, was added dropwise to a well-stirred solution of 2,2'-dihydroxybiphenyl(**1**) (0.930 g, 0.005 mole) and TEA (1.4 g, 0.01 mole) in dry THF (20 mL) at 0°C. After the addition, the temperature of the reaction mixture was slowly raised and kept at 25–30°C for 1 h. The reaction progress was monitored by TLC. The mixture was filtered to remove triethylamine hydrochloride and the filtrate was rota-evaporated. The residue-6-chlorodibenzo[d,f][1,3,2]dioxaphosphepine (**2**) was used for the next step.

To the intermediate **2** in dry THF (20 mL), 1,4-diaminobenzene (0.548 g, 0.05 mole) was added at 10°C in the presence of TEA at nitrogen atmosphere in dry THF. After the addition,

the reaction mixture was brought to 30–40°C and stirred for 2 h to complete the reaction. It was treated with alkyl/aryl azides (0.01 mole) in THF at 50–60°C and stirred for 4 h to complete the reaction. The progress of the reaction was monitored by TLC (ethyl acetate:hexane, 2:8) analysis. After completion of the reaction, solvent was removed in a rota-evaporator to get crude products. The residue was purified by column chromatography on silicagel (80–120 mesh) using petroleum ether-ethylacetate (8:2) as eluant. It was recrystallized from 2-propanol to afford pure (5a–j).

N1,N4-Bis[6-(methylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5a). Yield 65%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.81–7.96 (20H, m, Ar-H), 3.78 (2H, s, NH), 0.88 (6H, s, CH₃); ¹³C NMR data: 131.01 (C-1, C-11), 117.61 (C-2, C-10), 127.5 (C-3, C-9), 112.5 (C-4, C-8), 120.0 (C-12, C-13), 151.6 (C-14, C-15), 132.0 (C'-1, C'-4), 114.5 (C'-2, C'-3, C'-5, C'-6), 21.4 (CH₃-N); ³¹P NMR data: δ 6.75; IR (KBr) cm⁻¹: 3385 (P–NH), 1208 (P=N), 1045 (N–C); LCMS *m/z*: 595 (M⁺ + 1); Anal. Calcd. for C₃₂H₂₈N₄O₄P₂: C, 64.65; H, 4.75; N, 9.42. Found C, 64.60; H, 4.68; N, 9.36.

N1,N4-Bis[6-(ethylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5b). Yield 68%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.88–7.90 (20H, m, Ar-H), 3.95 (2H, s, NH), 1.88–2.01 (4H, m, CH₂), 1.22 (6H, t, *J* = 11.5 Hz, -CH₃); ³¹P NMR data: δ 7.78; IR (KBr) cm⁻¹: 3280 (P–NH), 1225 (P=N), 1055 (N–C); LCMS *m/z*: 626 (M⁺ + 1); Anal. Calcd. for C₃₄H₃₂N₄O₄P₂: C, 65.59; H, 5.18; N, 9.00. Found C, 65.51; H, 5.09; N, 8.95.

N1,N4-Bis[6-(propylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5c). Yield 65%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.76–7.70 (20H, m, Ar-H), 4.51 (2H, s, NH), 1.60 (4H, t, *J* = 9.5 Hz, -CH₂-CH₂-CH₃), 1.33–1.34 (4H, m, CH₂-CH₂-CH₃), 0.98 (6H, t, *J* = 10.3 Hz, -CH₂-CH₂-CH₃); ³¹P NMR data: δ 5.25; IR (KBr) cm⁻¹: 3350 (P–NH), 1220 (P=N), 1010 (N–C); Anal. Calcd. for C₃₆H₃₆N₄O₄P₂: C, 66.46; H, 5.58; N, 8.61. Found C, 66.40; H, 5.51; N, 8.55.

N1,N4-Bis[6-(butylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5d). Yield 69%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.80–7.82 (20H, m, Ar-H), 5.25 (2H, s, NH), 1.51 (4H, t, *J* = 6.8 Hz, -CH₂-CH₂-CH₃), 1.23–1.40 (8H, m, CH₂-CH₂-CH₂-CH₃), 0.95 (6H, t, *J* = 10.9 Hz, -CH₂-CH₂-CH₂-CH₃); ³¹P NMR data: δ 8.10; IR (KBr) cm⁻¹: 3410 (P–NH), 1265 (P=N), 1025 (N–C); Anal. Calcd. for C₃₈H₄₀N₄O₄P₂: C, 67.25; H, 5.94; N, 8.26. Found C, 67.20; H, 5.86; N, 8.18.

N1,N4-Bis[6-(vinylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5e). Yield 73%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.45–7.52 (20H, m, Ar-H), 5.05 (2H, t, *J* = 11 Hz, CH=CH₂), 4.22 (4H, d, *J* = 5.3 Hz, CH₂=CH), 3.82 (2H, s, NH); ¹³C NMR data: 128.0 (C-1, C-11), 118.7 (C-2, C-10), 127.4 (C-3, C-9), 113.1 (C-4, C-8), 125.7 (C-12, C-13), 151.0 (C-14, C-15), 145.0 (N-CH), 117.4 (CH=CH₂), 132.2 (C'-1, C'-4), 114.8 (C'-2, 3, 5); ³¹P NMR data: δ 6.59; IR (KBr) cm⁻¹: 3392 (P–NH), 1209 (P=N), 1081 (N–C); LCMS *m/z*: 618 (M⁺); Anal. Calcd. for C₃₄H₂₈N₄O₄P₂: C, 66.02; H, 4.56; N, 9.06. Found C, 65.96; H, 4.51; N, 9.01.

N1,N4-Bis[6-(isopropylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5f). Yield 70%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.91–7.79 (20H, m, Ar-H),

Table 1

DPPH radical scavenging activity of 5a–j.

Compound	IC ₅₀ (μg/mL)
5a	19
5b	19
5c	14
5d	13
5e	25
5f	.13
5g	16
5h	15
5i	13
5j	11
BHT	72.50

4.35 (2H, s, NH), 2.81–2.98 (2H, m, CH), 1.16 (12H, d, *J* = 10.2 Hz, CH-CH₃); ¹³C NMR data: 131.2 (C-1, C-11), 116.3 (C-2, C-10), 130.3 (C-3, C-9), 113.0 (C-4, C-8), 126.3 (C-12, C-13), 154.0 (C-14, C-15), 132.0 (C'-1, C'-4), 115.6 (C'-2, C'-3, C'-5, C'-6), 30.6 (CH-N), 16.6 (CH₃); ³¹P NMR data: δ 4.25; IR (KBr) cm⁻¹: 3370 (P–NH), 1216 (P=N), 1043 (N–C); Anal. Calcd. for C₃₆H₃₆N₄O₄P₂: C, 66.46; H, 5.58; N, 8.61. Found C, 66.40; H, 5.51; N, 8.56.

N1,N4-Bis[6-(isobutylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5g). Yield 72%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.79–7.81 (20H, m, Ar-H), 4.18 (2H, s, NH), 1.52–1.91 (2H, m, CH-CH₂), 1.28 (4H, d, *J* = 5.4 Hz, CH₂-CH), 1.15 (12H, t, *J* = 10.2 Hz, CH-CH₃); ¹³C NMR data: 131.6 (C-1, C-11), 118.7 (C-2, C-10), 128.4 (C-3, C-9), 114.8 (C-4, C-8), 125.7 (C-12, C-13), 155.0 (C-14, C-15), 127.4 (C'-1, C'-4), 117.3 (C'-2, C'-3, C'-5, C'-6), 35.0 (CH), 29.0 (N-CH₂), 19.2 (CH-CH₃); ³¹P NMR data: δ 9.20; IR (KBr) cm⁻¹: 3357 (P–NH), 1206 (P=N), 1058 (N–C); LCMS *m/z*: 678 (M⁺); Anal. Calcd. for C₃₈H₄₀N₄O₄P₂: C, 67.25; H, 5.94; N, 8.26. Found C, 67.19; H, 5.90; N, 8.21.

N1,N4-Bis[6-(allylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5h). Yield 71%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.80–7.99 (20H, m, Ar-H), 5.71 (2H, m, CH), 5.02–5.10 (4H, m, CH₂), 4.10 (2H, s, NH), 2.10 (4H, d, *J* = 5.2 Hz, N-CH₂); ³¹P NMR data: δ 9.10; IR (KBr) cm⁻¹: 3290 (P–NH), 1210 (P=N), 1029 (N–C); Anal. Calcd. for C₃₆H₃₂N₄O₄P₂: C, 66.87; H, 4.99; N, 8.66. Found C, 66.80; H, 4.92; N, 8.60.

N1,N4-Bis[6-(benzylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5i). Yield 75%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.80–7.96 (30H, Ar-H), 3.75 (2H, s, NH), 2.01 (4H, s, CH₂-Ar); ¹³C NMR data: 128.4 (C-1, C-11), 118.7 (C-2, C-10), 127.4 (C-3, C-9), 114.8 (C-4, C-8), 125.7 (C-12, C-13), 155.0 (C-14, C-15), 136.2 (C'-1, C'-4), 117.3 (C'-2, C'-3, C'-5, C'-6), 140.1 (C''-1), 130.1 (C''-2, C''-6), 128.2 (C''-3, C''-5), 125.8 (C''-4), 28.09 (CH₂-Ar); ³¹P NMR data: δ 4.94; IR (KBr) cm⁻¹: 3390 (P–NH), 1244.9 (P=N), 1045 (N–C); LCMS *m/z*: 746 (M⁺); Anal. Calcd. for C₄₄H₃₆N₄O₄P₂: C, 70.77; H, 4.86; N, 7.50. Found C, 70.71; H, 4.81; N, 7.45.

N1,N4-Bis[6-(nitrobenzylimino)-6λ⁵-dibenzo[d,f][1,3,2]dioxaphosphepin-6-yl]-1,4-benzene-diamine (5j). Yield 71%, viscous liquid; ¹H NMR (DMSO-*d*₆): δ 6.80–7.92 (28H, m,

Table 2
Nitric oxide scavenging activity of **5a–j**.

Name of the compound	IC ₅₀ (μg/mL)
5a	20
5b	22
5c	35
5d	29
5e	55
5f	24
5g	25
5h	22
5i	26
5j	14
BHT	357.14

Ar-H), 4.20 (2H, s, NH), 2.15 (4H, s, Ar-CH₂); ³¹P NMR data: δ 5.25; IR (KBr) cm⁻¹: 3390 (P–NH), 1225 (P=N), 1020 (N–C); LCMS *m/z*: 808 (M⁺ + 1); Anal. Calcd. for C₄₄H₃₄N₆O₈P₂: C, 63.16; H, 4.10; N, 10.04. Found C, 63.11; H, 4.05; N, 9.99.

ANTIOXIDANT ACTIVITY

DPPH radical scavenging activity. The hydrogen or electron donation abilities of title compounds were measured from the bleaching of the purple color methanol solution of DPPH [9]. This spectrophotometric assay uses the stable radical DPPH as a reagent. One milliliter of various concentrations of the title compounds (20, 40, 60, 80, and 100 μg/mL) in methanol were added to 4 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm.

Table 3
Antibacterial activity of **5a–j**.

Compd.	Zone of inhibition/mm					
	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>		
	100 (ppm ^a)	50 (ppm ^a)	25 (ppm ^a)	100 (ppm ^a)	50 (ppm ^a)	25 (ppm ^a)
5a	11	8	6	12	8	4
5b	8	6	–	10	7	5
5c	10	8	6	12	8	4
5d	6	5	4	12	6	6
5e	14	9	5	14	12	8
5f	13	11	8	13	11	7
5g	7	4	–	9	8	4
5h	10	8	5	10	6	5
5i	12	10	8	10	6	4
5j	11	8	5	12	8	6
^b <i>Penicillin</i>	9	6	–	12	8	–

^a In DMF^b Reference compound.**Table 4**Antifungal activity^a of **5a–j**.

Compd.	Zone of inhibition/mm					
	<i>Aspergillus niger</i>			<i>Helminthosporium oryzae</i>		
	100 (ppm ^a)	50 (ppm ^a)	25 (ppm ^a)	100 (ppm ^a)	50 (ppm ^a)	25 (ppm ^a)
5a	9	7	5	11	6	5
5b	10	8	4	11	9	5
5c	12	9	6	12	10	7
5d	11	10	8	14	10	4
5e	10	7	5	12	8	7
5f	11	5	3	12	10	9
5g	8	6	4	9	8	4
5h	9	8	6	11	9	5
5i	12	11	9	13	12	8
5j	8	9	6	9	7	4
^b <i>Griseofulvin</i>	12	10	5	12	10	5

^a In DMF.^b Reference compound.

The antioxidant activity of these compounds was expressed as IC₅₀ (Inhibition concentration, 50%) of **5j** which showed highest DPPH scavenging activity with 11 μg/mL when compared with other compounds (Table 1). The percent of inhibition of free radical production from DPPH was calculated by using the following equation. Butylated hydroxyl toluene (BHT) was used as a standard reference compound.

$$I = \frac{[(A_{\text{control}}) - (A_{\text{sample}})]}{\text{Blank}} \times 100 \quad (1)$$

where, A_{control} is absorbance of the control.

Control reaction containing the entire reagent except the test compound.

A_{sample} is absorbance of the test compound.

Nitric oxide scavenging activity. Nitric oxide scavenging activity was measured by slightly modified methods of Green *et al.* and Marcocci *et al.* [10]. Nitric oxide radicals were generated from sodiumnitroprusside. One milliliter of sodiumnitroprusside (mM) and 1.5 mL of phosphate buffer saline (0.2M, pH 7.4) were added to the different concentrations (20, 40, 60, 80, and 100 μg) of the extract and incubated for 150 min at 25°C. After incubation, 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% Sulfanilamide, 2% of H₃PO₄ and 0.1% naphthylethylene diamine dihydrochloride).

Absorbance was measured at 546 nm. **5j** showed highest DPPH scavenging activity with 14 mg/mL when compared with other compounds (Table 2). Butylated

hydroxy toluene was added as a standard. The percent of inhibition (I %) was calculated by using the following equation:

$$I = \frac{[(A_{\text{control}}) - (A_{\text{sample}})]}{\text{Blank}} 100 \quad (2)$$

Antibacterial activity. Antibacterial activity of all the title compounds (**5a-j**) was assayed [11] against *Staphylococcus aureus* ATCC-25923 (Gram positive) and *Escherichia coli* ATCC-25922 (Gram-negative) at three different concentrations (100, 50, and 25 ppm) in DMF (Table 3). The compounds were diluted in DMF for bioassay. Solvent control was included although no antibacterial activity has been noted in the solvent employed. Penicillin G (Hi-media) controls (20 $\mu\text{g}/\text{mL}^{-1}$) were included to compare with compounds (**5a-j**). All samples were tested in triplicate and average results were recorded.

Antifungal activity. The compounds (**5a-j**) were screened for their antifungal activity (Table 4) against *Aspergillus niger* and *Helminthosporium oryzae* species along with standard fungicide Griseofulvin at three different concentrations (100, 50, and 25 ppm) in DMF [12]. All the compounds (**5a-j**) exhibited moderate to high antifungal activity when compared with that of the reference compound. The majority of the compounds exhibited high activity against fungi.

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