Synthesis and Antioxidant Properties of New Substituted 8-Methyl-6-phenyl-5,6-dihydro-4*H*-1,3,2-benzodioxaphosphocine-2-oxide Derivatives

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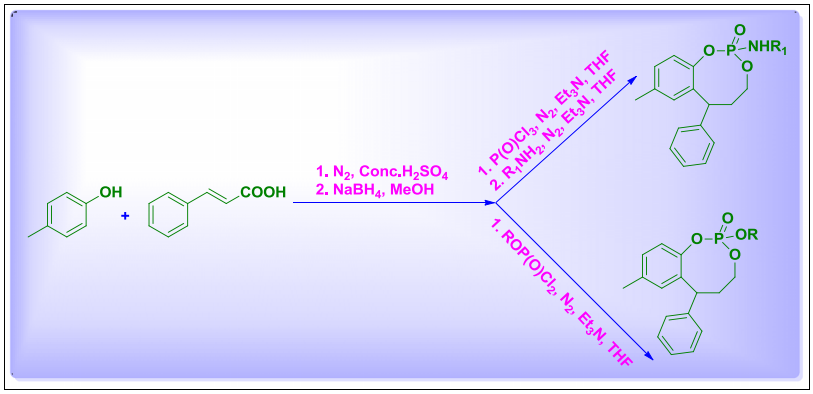
Additional Supporting Information may be found in the online version of this article.
Received October 17, 2015
DOI 10.1002/jhet.2638
Published online 00 Month 2016 in Wiley Online Library (wileyonlinelibrary.com).



A new route for the synthesis of substituted 8-methyl-6-phenyl-5,6-dihydro-4*H*-1,3,2-benzodioxaphosphocine-2-oxide derivatives has been developed by using cinnamic acid and *p*-cresol via condensation, reduction, and followed by phosphorylation steps. The title compounds were characterized by IR, ^{1}H, ^{13}C, ^{31}P, and mass spectral studies and elemental analysis. The title compounds have been investigated for their antioxidant activity with respect to their IC_{50} values using 2,2-diphenyl-1-picrylhydrazyl, NO radical scavenging activities, and reducing power assay. The results obtained from the aforementioned methods revealed that 2-phenylamino derivatives have shown greater free radical scavenging activity when compared with those of the phenoxy derivatives and is attributed to the presence of secondary amino group, which is able to produce free radicals easily.

J. Heterocyclic Chem., **00,** 00 (2016).

INTRODUCTION

Oxidation reactions generates reactive oxygen species (ROS) in regular metabolisms consisting of both free radicals ($OH^{\cdot}$), superoxide anions (O_2^{-}), and non free radical compounds like hydrogen peroxide (H_2O_2), organic peroxide (ROOH), ozone (O_3), and singlet oxygen ($^{1}O_2$). These ROS species induce oxidative stress and are also responsible for diabetes, cardiovascular, and many of today's diseases [1–3]. They play key role in DNA damage [4], mutations, cellular injury, oncogenesis, and aging process [5,6]. In addition, the ROS are considered to cause cancer, and several neurodegenerative diseases such as Alzheimer's, Parkinson's, Down's syndrome, inflammation, viral infections, and various other digestive disorders such as ulcers and gastrointestinal problems are the results of ROS generation in the living systems [7,8].

Focused on the chemistry of organophosphorus heterocyclics, attention has been increased from past three decades because of their unique physicochemical properties [9] and potential biological activities [10–13]. Majority of organophosphorus heterocyclic systems include structural moieties with P–O and P–N bonds like cyclophosphamide, and its derivatives as proven antitumor agents are the earliest examples [14]. Cyclic phosphorus-containing hexapyranose analog modified at the anomeric carbon are the regulators in the carbohydrate-linked life process [15]. 1,4-Dihydropyridine-5-cyclic phosphonate derivatives have antihypertensive activity [16]. Cyclophosphorylation of polyphenols and tetrahydroxydinaphthylmethanes by diamidoarylphosphites has generated a new class of macrophosphocyclic systems, specifically phosphocines [17,18].

Phosphorus containing heterocycles serve as active catalysts in asymmetric and natural product synthesis [19]. Also their role has been expanded in the field of fire and flame proofing as flame retardants such as Melamine salt of pentaerythritol phosphite [20] and hexa (phosphaphenanthrene aminophenoxyl)-cyclotriphosphazene [21]. They also act as metal chelators [22] and corresponding metal complexes that serve as highly active catalysts in organocatalysis [19].

The importance of phosphorus containing heterocycles inspired to design and synthesize a new substituted 8-methyl-6-phenyl-5,6-dihydro-4*H*-1,3,2-benzodioxaphosphocine-2-oxide derivatives and study their antioxidant activity.

RESULTS AND DISCUSSION

Chemistry. *p*-Cresol (**1**) and cinnamic acid (**2**) were condensed in the presence of con. H_2SO_4 under nitrogen atmosphere at 120°C for a period of 6 h to afford 3,4-dihydro-6-methyl-4-phenyl-2*H*-benzopyran-2-one (**3**). Reduction of **3** with $NaBH_4$ in methanol produced 2-(3-hydroxy-1-phenylpropyl)-4-methylphenol (**4**). Phosphorylation of **4** with $P(O)Cl_3$ afforded the monochloride (**5**), which was subsequently condensed with amines to obtain the 2-amino derivatives of the title compounds **6a–j**. Reaction of **4** directly with $ROP(O)Cl_2$ led to the formation of the corresponding 2-aryloxy derivatives **7a–d** (the spectrum of compound **7a** is described in the Supporting Information). The synthetic route is presented in Scheme 1.

Almost all the title compounds are obtained in moderate to good yields (**56–73**). Among them compounds **7a–d** are somehow produced in slightly higher yields compared with **6a–j**. This may be attributed to the more effective nucleophilic cyclocondensation of compound **4** at the electrophilic phosphorous of $ROP(O)Cl_2$ in synthetic steps of **7a–d** compared with that of the synthetic step leading **6a–j**.

Further, the variations in the yields of compounds **6a–j** are related to their chemical structural variations. The substituents having electron withdrawing groups (–F, $–NO_2$, –Cl, –Br) are decreasing the electron density on the N atom of amino group, and thus, the ease of chloride displacement by the nitrogen nucleophilic substrates becomes difficult as this results in lowering the product yields. This effect is exactly reverse in case of electron releasing groups ($–CH_3$, $–OCH_3$, $–N(Me)_2$), which facilitate cyclization of the phosphorus atom of **5** and produced relatively high yields. The results are incorporated in Table 1.

ANTIOXIDANT ACTIVITY

The substituted 1,3,7,10-dioxadiazaphosphacyclotridecinones (**I**) and 1,3,2-diazaphosphole-1-oxides (**II**) are structurally important macrophosphorus heterocyclic molecules, which are active antioxidants [23,24] shown in Figure 1.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity. Form the results, the order of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of compounds: **6i** (IC_{50} 52 μg/mL) > **6j** (IC_{50} 54 μg/mL) > **6f** (IC_{50} 56 μg/mL) > **6e** (IC_{50} 57 μg/mL) > ascorbic acid **6k** (IC_{50} 62 μg/mL) > **6d** (IC_{50} 65 μg/mL) as represented in Figure 2.

Nitric oxide (NO) scavenging activity. Form the results (Fig. 3), the order of NO scavenging activity of compounds: **6i** (IC_{50} 54 μg/mL) > **6j** (IC_{50} 55 μg/mL) > **6e** (IC_{50} 57 μg/mL) > **6f** (IC_{50} 58 μg/mL) > ascorbic acid **6k** (IC_{50} 66 μg/mL) > **6h** (IC_{50} 68 μg/mL).

Reducing power assay. According to the results obtained (Fig. 4), **6e** and **6j** showed highest reducing power (IC_{50} of

Scheme 1. Synthesis of 8-methyl-6-phenyl-5,6-dihydro-4*H*-1,3,2-benzodioxaphosphocine 2-oxide derivatives **6a–j** and **7a–d**.

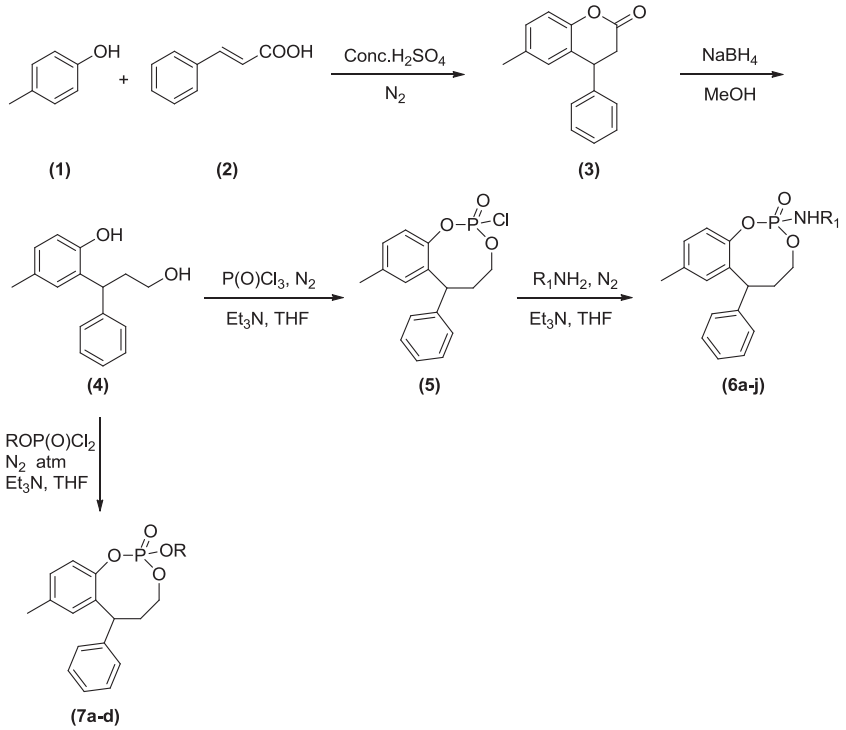
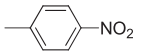
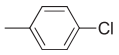
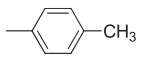
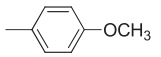
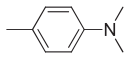
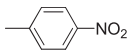
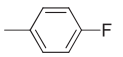
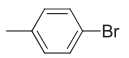
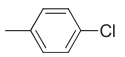
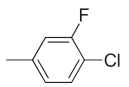
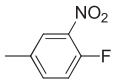


Table 1

Synthesis of compounds **6a–j** and **7a–d**.

Compound	R	R_1	Yield (%)
7a		—	**73**
7b		—	**70**
7c		—	**72**
7d		—	**71**
6a	—		**63**
6b	—		**67**
6c	—		**64**
6d	—		**66**
6e	—		**69**
6f	—		**60**
6g	—		**61**
6h	—		**61**
6i	—		**58**
6j	—		**56**

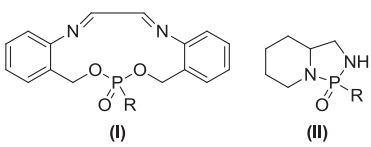


Figure 1. Important antioxidant phosphorus heterocycles.

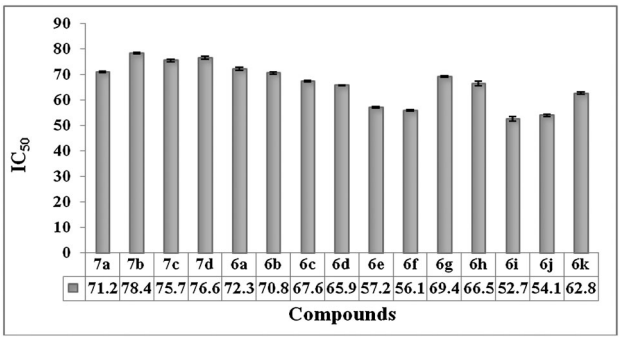


Figure 2. DPPH radical scavenging activity of compounds **6a–j**, **7a–d**, and **6k** = ascorbic acid.

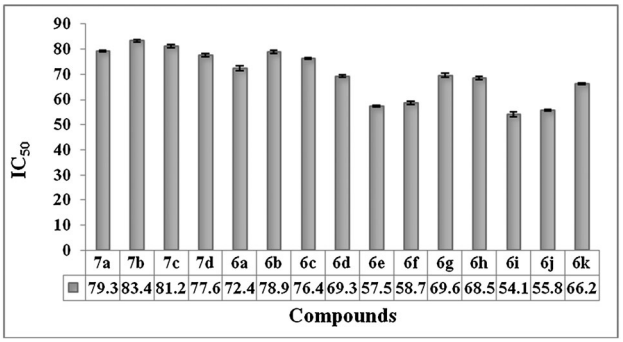


Figure 3. NO scavenging activity of compounds **6a–j**, **7a–d**, and **6k** = ascorbic acid.

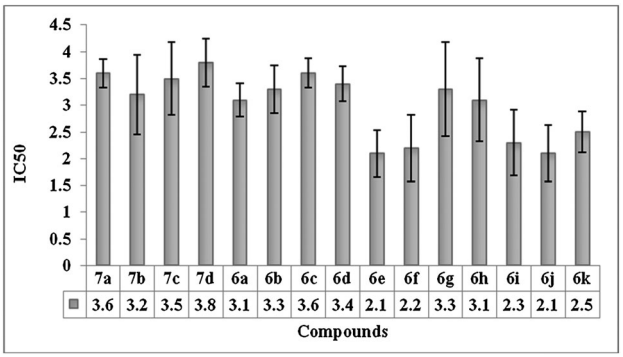


Figure 4. Reducing power assay of compounds **6a–j**, **7a–d**, and **6k** = ascorbic acid.

2.1 μg/mL) when compared with other compounds. The remaining compounds exhibited reducing power activity in the following order: **6f** (IC_{50} 2.2 μg/mL) > **6i** (IC_{50} 2.3 μg/mL) > ascorbic acid **6k** (IC_{50} 2.5 μg/mL).

The results from all the methods showed that 2-phenylamino derivatives **6a–j** in general have shown greater free radical scavenging activity when compared with those of the phenoxy derivatives **7a–d**. The corresponding higher activity in phosphoramidate derivatives (**6a–j**) is due to the presence of secondary amines and

can undergo homolytic cleavage easily to form the corresponding free radicals, which can inhibit the active free radicals developed in the metabolic pathways and is not that much feasible in case of phosphate derivatives (**7a–d**).

In particular, compounds **6e**, **6f**, **6i**, and **6j** with –F, –Cl, and $–NO_2$ substituents exhibits much higher antioxidant activity compared with the reference standard ascorbic acid (**6k**) because the high electron withdrawing nature of these substituents facilitates neutralization of free radicals. It is noteworthy that compound **6e** with $–NO_2$ substituent exhibited very high activity in reducing power assay compared with other methods. Thus, a new class of antioxidants has been developed.

Experimental. All chemicals were procured from Sigma-Aldrich (Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India) and used without further purification. The melting points were determined in open capillary tubes on a Mel-Temp apparatus (Tempo Instruments and Equip (Pvt.) Ltd., Mumbai, India) and are uncorrected. All the IR spectra of the title compounds were recorded on Bruker Alpha-Eco attenuated total reflection–Fourier transform infrared interferometer with single reflection sampling module equipped with ZnSe crystal (Labindia Analytical Instruments Pvt. Ltd. Hyderabad, India). ^1H and ^{13}C NMR spectra were recorded on a Bruker instrument (Labindia Analytical Instruments Pvt. Ltd. Hyderabad, India) at 500 and 125 MHz in DMSO-d_6 using TMS as internal standard. ^{31}P NMR (200 MHz) was taken in DMSO-d_6 using 85% H_3PO_4 as external standard. Mass spectra and the elemental analysis were obtained on LCMS-2010A Shimadzu spectrometer (Inkarp Instruments Pvt Ltd., Hyderabad) and EA 1112 Thermo Finnigan instrument (Courtaboeuf, France), respectively, at the University of Hyderabad, India.

Synthesis of 3,4-dihydro-6-methyl-4-phenyl-2*H*-benzopyran-2-one (3). Trans cinnamic acid (**2**, 5 g, 33.78 mmol) was taken in a 250-mL three-necked round bottomed flask equipped with a mechanical stirrer, thermocouple, and nitrogen inlet. Preheated Para-Cresol (**1**, 3.82 g, 135.43 mmol) in a water bath at 60°C was added to the cinnamic acid (**2**) followed by conc. H_2SO_4 (0.64 mL, 12.16 mmol). The reaction was immediately heated to 120°C, and the mixture was stirred for a period of 6 h and cooled to 100°C. It was taken into a pre-warmed separating funnel (250 mL), and the bottom layer containing the H_2SO_4 was removed. Toluene–water mixture (6:1; 20 mL) and potassium carbonate solution (5 mL) were added to the separating funnel containing the crude product and then mixed. The organic layer was separated and concentrated to half of its original volume. To this, nearly double the volume of isopropanol was added, and the total volume of the mixture was reduced to the level of added volume of isopropanol. This was repeated for three times. Then the mixture was cooled to 30–40°C and rapidly stirred. It resulted in the separation of a crystalline product from the mixture. The reaction mixture was cooled to 0–5°C for 30 min and filtered. The resulting solid was washed with isopropanol until the compound is colorless and pure, which is confirmed by determining its melting point of 83–85°C.

Synthesis of 2-(3-hydroxy-1-phenylpropyl)-4-methylphenol (4). Two grams of compound **3** was taken in a 100-mL round bottomed flask. To this, methanol (5 mL) was added and cooled to 0°C. Compound **3** was reduced with $NaBH_4$. After completion of reaction, as monitored by TLC, methanol was removed on rota evaporator. The obtained crude product was taken in a beaker, and cold water was added. On stirring the mixture, a white solid separated. It was filtered, dried, and recrystallized to obtain pure compound **4**.

Synthesis of compounds 7a–d. Compound **4** (1 mmol) was taken in a two-necked 50-mL round bottom flask. One neck was fitted with N_2 inlet; through the other neck, 5 ml of dry THF was added followed by Et_3N (2 mmol). The contents were stirred in ice bath for 5 min. After that, phenylphosphorodichloridate (1 mmol) dissolved in 5 mL of dry THF was added dropwise for a period of 30 min at 0°C. After completion of reaction, as monitored by TLC, the mixture was filtered to remove triethylamine hydrochloride. THF from the filtrate was evaporated under reduced pressure. The crude product obtained was columned on silica gel (60–120 mesh) and eluted with ethylacetate/hexane (1:4) to obtain the pure compound **7a**. Other compounds **7b–d** were synthesized by following the aforementioned procedure.

Synthesis of compounds 6a–j. Compound **4** (1 mmol) was taken in a two-necked 50-mL round bottomed flask. One neck was fitted with N_2 inlet, and through the other neck, 5 mL of dry THF followed by Et_3N (2 mmol) were added. The contents were stirred in ice bath for 5 min. After that, $P(O)Cl_3$ (1 mmol) in dry THF was added dropwise for a period of 30 min at 0°C. Completion of the reaction was monitored by TLC. The mixture was filtered to remove triethylamine hydrochloride. The solvent from the filtrate was evaporated under reduced pressure to obtain the crude product. It was columned by using silica gel (60–120 mesh) with ethylacetate/hexane (1:5) as eluent to obtain the intermediate monochloride (**5**).

To a stirred solution of aniline (1 mmol) and Et_3N (2 mmol) in dry THF (5 mL), the intermediate monochloride (**6**, 1 mmol) in dry THF was added dropwise at 0°C. After addition, the temperature was raised to 40–50°C and stirred for 4–5 h. After completion of the reaction as indicated by TLC, the mixture was filtered to remove triethylamine hydrochloride, and the solvent from the filtrate was evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (60–120 mesh) with ethyl acetate/hexane (1:4) as eluent to afford pure compound **6a**. Compounds **6a–j** were prepared by the same procedure.

Physical and spectral data of compounds 7a–d and 6a–j.

8-Methyl-2-phenoxy-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocine-2-oxide (7a). White solid, mp: 116–118°C; IR (ZnSe, cm^{-1}): ν 3048 (aromatic C–H), 1295 (P=O), 944 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.25–6.97 (m, 13H, Ar–H), 4.41–4.38 (m, 1H, CH), 3.34–3.29 (m, 2H, OCH_2), 2.15–2.10 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 152.6, 145.3 (2C), 131.2 (2C), 130.5 (2C), 128.0 (2C), 127.9 (2C), 126.9 (2C), 125.5 (2C), 121.6, 115.0 (2C), 59.2, 39.3 (2C), 20.4; ^{31}P NMR (200 MHz, DMSO-d_6): δ 22.42; HRMS [m/z]: 381.1827 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{21}O_4P$: C, 69.47; H, 5.56; Found C, 69.38; H, 5.52.

8-Methyl-2-(4-nitrophenoxy)-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocine-2-oxide (7b). Pale yellow solid, mp: 104–106°C; IR (ZnSe, cm^{-1}): ν 3035 (aromatic C–H), 1278 (P=O), 956 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10–6.98 (m, 12H, Ar–H), 4.39–4.36 (m, 1H, CH), 3.35–3.29 (m, 2H, OCH_2), 2.17–2.09 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 154.2, 145.5 (2C), 141.2, 131.3 (2C), 130.7, 128.3 (2C), 128.1 (2C), 126.5, 125.4 (2C), 121.7, 116.1 (2C), 61.2, 39.4 (2C), 20.5; ^{31}P NMR (200 MHz, DMSO-d_6): δ 18.65; LCMS [m/z]: 426.12 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{20}NO_6P$: C, 62.12; H, 4.74; N, 3.29; Found C, 62.05; H, 4.68; N, 3.21.

2-(4-Chlorophenoxy)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocine-2-oxide (7c). White solid, mp: 118–120°C; IR (ZnSe, cm^{-1}): ν 3045 (aromatic C–H), 1287 (P=O), 946 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.43–6.92 (m, 12H, Ar–H), 4.29–4.27 (m, 1H, CH), 3.31–3.27 (m, 2H, OCH_2), 2.18–2.13 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 150.2, 143.6 (2C), 131.2 (2C), 129.7, 128.2 (2C), 127.6 (2C), 126.3 (2C), 125.1 (2C), 121.8 (2C), 116.3, 115.2, 60.8, 40.1, 39.7, 20.6; ^{31}P NMR (200 MHz, DMSO-d_6): δ 18.73; LCMS [m/z]: 415.14 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{20}ClO_4P$: C, 63.70; H, 4.86; Found C, 63.65; H, 4.78.

2-(2-Chlorophenoxy)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocine-2-oxide (7d). White solid, mp: 117–119°C; IR (ZnSe, cm^{-1}): ν 3042 (aromatic C–H), 1285 (P=O), 941 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.42–6.94 (m, 12H, Ar–H), 4.30–4.27 (m, 1H, CH), 3.33–3.27 (m, 2H, OCH_2), 2.19–2.13 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 150.5, 143.2 (2C), 130.9 (2C), 129.3, 128.0 (2C), 127.1 (2C), 126.6 (2C), 125.3 (2C), 121.8 (2C), 115.2 (2C), 60.6, 40.5, 39.2, 20.7; ^{31}P NMR (200 MHz, DMSO-d_6): δ 18.75; LCMS [m/z]: 415.08 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{20}ClO_4P$: C, 63.70; H, 4.86; Found C, 63.64; H, 4.81.

8-Methyl-N,6-diphenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocin-2-amine-2-oxide (6a). White solid, mp: 124–126°C; IR (ZnSe, cm^{-1}): ν 3335 (NH), 1265 (P=O), 937 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.41–6.72 (m, 13H, Ar–H), 5.51 (brs, 1H, NH), 4.31–4.27 (m, 1H, CH), 3.35–3.27 (m, 2H, OCH_2), 2.45–2.32 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 146.2 (2C), 142.3, 132.7, 131.2 (2C), 130.1, 129.2 (2C), 128.7 (2C), 127.8 (2C), 126.3, 120.1, 119.2, 118.7 (2C), 61.2, 40.2, 39.4, 20.7; ^{31}P NMR (200 MHz, DMSO-d_6): δ 18.73; LCMS [m/z]: 380.32 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{22}NO_3P$: C, 69.65; H, 5.84; N, 3.69; Found C, 69.61; H, 5.78; N, 3.65.

8-Methyl-6-phenyl-2-(4-toluidino)-5,6-dihydro-4H-1,3,2λ^5-benzodioxaphosphocin-2-one (6b). White solid, mp: 120–122°C; IR (ZnSe, cm^{-1}): ν 3362 (NH), 1271 (P=O), 943 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.39–6.63 (m, 12H, Ar–H), 5.43 (brs, 1H, NH), 4.36–4.32 (m, 1H, CH), 3.34–3.28 (m, 2H, OCH_2), 2.35–2.15 (m, 8H, O–CH_2–$\underline{CH_2}$– and 2XCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 146.3 (2C), 141.7, 133.1, 131.5 (2C), 130.2, 129.3 (2C), 128.5 (2C), 127.7 (2C), 126.5, 120.3, 119.5, 118.9 (2C), 55.6, 40.3, 39.6, 20.8 (2C); ^{31}P NMR (200 MHz, DMSO-d_6): δ 19.21; LCMS [m/z]: 394.26 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{23}H_{24}NO_3P$: C, 70.22; H, 6.15; N, 3.56; Found C, 70.18; H, 6.09; N, 3.52.

2-(4-Methoxyanilino)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2λ^5-benzodioxaphosphocin-2-one (6c). White solid, mp: 120–122°C; IR (ZnSe, cm^{-1}): ν 3278 (NH), 1263 (P=O), 956 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.41–6.62 (m, 12H, Ar–H), 5.47 (brs, 1H, NH), 4.35–4.29 (m, 1H, CH), 3.72 (s, 3H, OCH_3), 3.34–3.27 (m, 2H, OCH_2), 2.31–2.18 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 154.2, 146.4 (2C), 134.7, 132.2, 131.3 (2C), 129.2 (2C), 128.1 (2C), 126.5, 125.7, 119.2, 117.3 (2C), 115.6 (2C), 62.3, 54.8, 40.2 (2C), 20.7; ^{31}P NMR (200 MHz, DMSO-d_6): δ 20.12; LCMS [m/z]: 410.43 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{23}H_{24}NO_4P$: C, 67.47; H, 5.91; N, 3.42; Found C, 67.42; H, 5.85; N, 3.37.

2-[4-(Dimethylamino)anilino]-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2λ^5-benzodioxaphosphocin-2-one (6d). White solid, mp: 126–128°C; IR (ZnSe, cm^{-1}): ν 3314 (NH), 1245 (P=O), 973 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.38–6.67 (m, 12H, Ar–H), 5.56 (brs, 1H, NH), 4.31–4.24 (m, 1H, CH), 3.4–3.1 (s, 6H, N(CH_3)$_2$), 3.37–3.26 (m, 2H, OCH_2), 2.28–2.19 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 150.6, 146.2 (2C), 132.3 (2C), 130.7 (2C), 129.6 (2C), 128.4 (2C), 127.2, 125.3, 119.8 (2C), 117.3 (2C), 114.2 (2C), 59.3, 41.5 (2C), 40.3, 39.2, 20.7; ^{31}P NMR (200 MHz, DMSO-d_6): δ 18.76; LCMS [m/z]: 423.24 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{24}H_{27}N_2O_3P$: C, 68.23; H, 6.44; N, 6.63; Found C, 68.18; H, 6.41; N, 6.57.

8-Methyl-2-(4-nitroanilino)-6-phenyl-5,6-dihydro-4H-1,3,2λ^5-benzodioxaphosphocin-2-one (6e). Pale yellow solid, mp: 124–126°C; IR (ZnSe, cm^{-1}): ν 3258 (NH), 1254 (P=O),

936 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.01–6.83 (m, 12H, Ar–H), 5.63 (brs, 1H, NH), 4.35–4.21 (m, 1H, CH), 3.36–3.27 (m, 2H, OCH_2), 2.26–2.18 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 149.3, 146.5 (2C), 137.3, 132.7, 131.2 (2C), 129.3 (2C), 128.5 (2C), 126.8 (2C), 124.9 (2C), 119.3, 117.6 (2C), 59.2, 40.7, 39.4, 20.6; ^{31}P NMR (200 MHz, DMSO-d_6): δ 19.27; LCMS [*m*/*z*]: 425.32 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{21}N_2O_5P$: C, 62.26; H, 4.99; N, 6.60; Found C, 62.21; H, 4.92; N, 6.56.

N-(4-Fluorophenyl)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocin-2-amine-2-oxide (6f). White solid, mp: 130–132°C; IR (ZnSe, cm^{-1}): *v* 3285 (NH), 1242 (P=O), 963 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.43–6.72 (m, 12H, Ar–H), 5.71 (brs, 1H, NH), 4.31–4.25 (m, 1H, CH), 3.32–3.25 (m, 2H, OCH_2), 2.28–2.21 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.2, 147.3 (2C), 139.5, 132.7, 131.3 (2C), 129.5 (2C), 128.2 (2C), 127.3, 126.8, 119.3, 117.5 (2C), 116.2 (2C), 57.2, 39.7 (2C), 20.7; ^{31}P NMR (200 MHz, DMSO-d_6): δ 21.07; LCMS [*m*/*z*]: 398.18 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{21}FNO_3P$: C, 66.49; H, 5.33; N, 3.52; Found C, 66.45; H, 5.31; N, 3.46.

N-(4-Bromophenyl)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocin-2-amine-2-oxide (6g). Pale yellow solid, mp: 126–128°C; IR (ZnSe, cm^{-1}): *v* 3345 (NH), 1263 (P=O), 947 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.43–6.62 (m, 12H, Ar–H), 5.62 (brs, 1H, NH), 4.32–4.29 (m, 1H, CH), 3.35–3.27 (m, 2H, OCH_2), 2.27–2.18 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 146.2 (2C), 141.7, 132.6 (2C), 132.1, 131.5 (2C), 129.2 (2C), 128.5 (2C), 127.3, 126.5, 119.8, 118.3 (2C), 116.5, 61.3, 40.2, 38.7, 21.2; ^{31}P NMR (200 MHz, DMSO-d_6): δ 20.17; LCMS [*m*/*z*]: 459.13 [M + 1]$^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{21}BrNO_3P$: C, 57.66; H, 4.62; N, 3.06; Found C, 57.62; H, 4.56; N, 2.98.

N-(4-Chlorophenyl)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocin-2-amine-2-oxide (6h). White solid, mp: 124–126°C; IR (ZnSe, cm^{-1}): *v* 3254 (NH), 1238 (P=O), 945 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.41–6.72 (m, 12H, Ar–H), 5.55 (brs, 1H, NH), 4.35–4.31 (m, 1H, CH), 3.37–3.32 (m, 2H, OCH_2), 2.25–2.19 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 145.7 (2C), 141.2, 132.7, 131.6 (2C), 130.2 (2C), 129.3 (2C), 128.7 (2C), 127.5 (2C), 125.9, 119.8, 117.2 (2C), 61.2, 40.4, 39.7, 20.8; ^{31}P NMR (200 MHz, DMSO-d_6): δ 18.71; LCMS [*m*/*z*]: 414.45 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{21}ClNO_3P$: C, 63.85; H, 5.11; N, 3.38; Found C, 63.81; H, 5.08; N, 3.35.

N-(4-Chloro-3-fluorophenyl)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2-benzodioxaphosphocin-2-amine-2-oxide (6i). White solid, mp: 132–134°C; IR (ZnSe, cm^{-1}): *v* 3316 (NH), 1262 (P=O), 956 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.41–6.58 (m, 11H, Ar–H), 5.62 (brs, 1H, NH), 4.33–4.28 (m, 1H, CH), 3.36–3.29 (m, 2H, OCH_2), 2.28–2.21 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 164.2, 147.5 (2C), 140.3, 132.1 (2C), 131.4 (2C), 129.7 (2C), 128.2 (2C), 127.5, 126.3, 118.5, 114.2, 110.3, 107.1, 61.3, 39.4 (2C), 20.7; ^{31}P NMR (200 MHz, DMSO-d_6): δ 19.87; LCMS [*m*/*z*]: 432.26 [M + 1] $^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{20}ClFNO_3P$: C, 61.19; H, 4.67; N, 3.24; Found C, 61.13; H, 4.62; N, 3.21.

2-(4-Fluoro-3-nitroanilino)-8-methyl-6-phenyl-5,6-dihydro-4H-1,3,2λ^5-benzodioxaphosphocin-2-one (6j). Pale yellow solid, mp: 128–130°C; IR (ZnSe, cm^{-1}): *v* 3263 (NH), 1245 (P=O), 939 (P–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.62–6.91 (m, 11H, Ar–H), 5.73 (brs, 1H, NH), 4.42–4.37 (m, 1H, CH), 3.31–3.25 (m, 2H, OCH_2), 2.35–2.27 (m, 5H, O–CH_2–$\underline{CH_2}$– and Ar–CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 146.2 (2C), 145.3, 137.1 (2C), 132.3, 131.4 (2C), 129.2 (2C), 128.6 (2C), 127.1, 126.3, 124.2, 119.3, 117.6, 110.4, 61.3, 40.2, 35.4, 21.6; ^{31}P NMR (200 MHz, DMSO-d_6): δ 19.65; LCMS [*m*/*z*]: 443.37 [M + 1]$^{+\cdot}$; *Anal*. Calcd for $C_{22}H_{20}FN_2O_5P$: C, 59.73; H, 4.56; N, 6.33; Found C, 59.68; H, 4.52; N, 6.29.

ANTIOXIDANT ACTIVITY

The antioxidant property of title compounds **7a–d** and **6a–j** were measured in terms of IC_{50} values by DPPH, NO radical scavenging, and reducing power assay methods.

DPPH radical scavenging activity. The free radical scavenging activity of compounds **7a–d** and **6a–j** against DPPH radical was performed in accordance with the method of Choi *et al*., wherein 85 μM of DPPH was added to a medium containing different target compounds [25]. 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).

$$\text{DPPH Radical Scavenging Activity}\,(\%) = \frac{[(A_{\text{Control}} - A_{\text{Test}})]}{(A_{\text{Control}})} \times 100$$

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

Nitric Oxide Scavenging Activity. Sodium nitroprusside (5 μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentration of test compounds dissolved in dioxane or methanol, and the tubes were incubated at 25°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*-napthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water) [25]. The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-napthylethylenediamine dihydrochloride was read at λ 546 nm. The experiment was repeated in triplicate.

$$\text{NO Radical Scavenging Activity } (\%) = \frac{[(A_{\text{Control}} - A_{\text{Test}})]}{(A_{\text{Control}})} \times 100$$

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

Reducing power assay. The reducing power of compounds **7a–d** and **6a–j** was determined according to the method of Oyaizu *et al.* [26]. The compounds having 50–100 μ*M* 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 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 in 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%.

CONCLUSION

A new synthetic route for the synthesis of new substituted 8-methyl-6-phenyl-5,6-dihydro-4*H*-1,3,2-benzodioxaphosphocine-2-oxide derivatives with O–P–O linkage has been developed. This is achieved from the commercially available less expensive chemicals cinnamic acid and *p*-cresol in a four-step method. The title compounds have been investigated for their antioxidant activity with respect to their IC_{50} values in three methods (DPPH, NO, and reducing power assays). Among the title compounds **6e**, **6f**, **6i**, and **6j** with **–F**, **–Cl**, and **–NO_2** substituents show high antioxidant activity in all methods.

Acknowledgments. We thank the Board of Research in Nuclear Sciences (BRNS), Mumbai, India for funding through the Research Project (F. No.: 37(2)/14/03/2014-BRNS/0149, Dated 25-04-2014).

REFERENCES

[1] Kohen, R.; Nyska, A. Toxicol Pathol 2002, 30, 620.
[2] Samarkoon, S. M. S.; Chandola, H. M.; Shukla, V. J. Int J Ayurveda Res 2011, 2, 23.
[3] Poorna, C. A.; Resmi, M. S.; Soniya, E. V. Agric Chem New York: InTech 2013 155.
[4] Kumar, V.; Lemos, M.; Sharma, M.; Shriram, V. Free Radicals and Antioxid 2013, 3, 55.
[5] Zahin, M.; Ahmad, I.; Aqil, F. Toxicol In Vitro 2010, 24, 1243.
[6] Kumar, M.; Kumar, S.; Kaur, S. Afr J Pharm Pharmacol 2011, 5, 421.
[7] Repetto, M. G.; Llesuy, S. F. Braz J Med Biol Res 2002, 35, 523.
[8] Surh, Y. J.; Ferguson, L. R. Mutat Res 2003, 1, 523.
[9] He, L. N.; Luo, Y. P.; Ding, M. W.; Lu, A. H.; Liu, X. P.; Wu, T.; Cai, F. Synth Commun 2002, 32, 1415.
[10] Quin, L. D. The Heterocyclic Chemistry of Phosphorus; John Wiley & Sons: New York, 1981; Vol 81.
[11] Cava, M. P.; Levinson, M. I. Tetrahedron 1985, 41, 5061.
[12] Cherkasov, R. A.; Kutyrev, G. A.; Pudovik, A. N. Tetrahedron 1985, 41, 2588.
[13] Rao, L. N.; Reddy, V. K.; Reddy, C. D. Heteroatom Chem 2000, 11, 323.
[14] Gilard, V.; Martino, R.; Malet-Martino, M.; Niemeyer, U.; Pohl, J. J Med Chem 1999, 42, 2542.
[15] 3Darrow, J. W.; Drueckhammer, D. G. J Org Chem 1994, 59, 2976.
[16] Morita, I.; Kunimoto, K.; Tsuda, M.; Tada, S. I.; Kise, M.; Kimura, K. Chem Pharm Bull 1987, 35, 4144.
[17] Vera, I. M.; Roman, V. M.; Maria, V. D.; Larisa, K. V.; Konstantin, A. L.; Mikhail, Y. A.; Dirk, W.; Ingmar, B.; Wolf, D. H.; Eduard, E. N. Tetrahedron 2003, 59, 1753.
[18] Vera, I. M.; Tatyana, Y. S.; Larisa, K. V.; Konstantin, A. L.; Mikhail, Y. A.; Sergei, O. A.; Andrei, I. D.; Wolf, D. H.; Eduard, E. N. Tetrahedron 2007, 63, 4162.
[19] Wang, C.; Han, Z. Y.; Luo, H. W.; Gong, L. Z. Org Lett 2010, 12, 2266.
[20] Chen, Y.; Wang, Q. Polym Adv Technol 2007, 18, 587.
[21] Jiang, P.; Gu, X.; Zhang, S.; Wu, S.; Zhao, Q.; Hu, Z. Ind Eng Chem Res 2015, 54, 2974.
[22] Amir, A.; Ezra, A.; Shimon, L. J. W.; Fischer, B. Inorg Chem 2014, 53, 7901.
[23] Naidu, K. R. M.; Kumar, M. A.; Dadapeer, E.; Babu, K. R.; Raju, C. N.; Ghosh, S. K. J Heterocyclic Chem 2011, 48, 317.
[24] Naidu, K. R. M.; Rao, P. V.; Raju, C. N.; Srinivasulu, K. Arch Pharm Chem Life Sci 2011, 344, 765.
[25] Choi, C. W.; Kim, S. C.; Hwang, S. S.; Choi, B. K.; Ahn, H. J.; Lee, M. Y.; Park, S. H.; Kim, S. K. Plant Sci 2002, 153, 1161.
[26] Oyaizu, M. Jpn J Nutr 1986, 44, 307.