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SYNTHESIS AND BIOLOGICAL ACTIVITY OF DIAZAPHOSPHOLO-IMINOPHOSPHORANE DERIVATIVES OF ZIDOVUDINE (AZT) THROUGH STAUDINGER REACTION

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ABSTRACT

Synthesis of a series of novel iminophosphorane derivatives of Zidovudine (AZT) was accomplished through Staudinger reaction. In the first step PBr_3 was reacted with various substituted phenolic and amino compounds in the presence of triethylamine (TEA) in dry tetrahydrofuran (THF) under nitrogen atmosphere followed by cyclisation of (±) 2-aminomethyl piperidine, afforded diazaphosphole P(III) intermediates (**3a-l**).They were further reacted with zidovudine to obtain iminophosphorane derivatives **4a-l**. They showed promising antioxidant activitiy. Their antioxidant activitiy was greatly influenced by the presence of different bio-active groups at phosphorus.

KEYWORDS

Diazaphospholo-imine derivatives of AZT, Staudinger Reaction, Antioxidant activity, Iminophosphoranes.

INTRODUCTION

The Staudinger reaction[1-3] reaction of tertiary phosphines with organic azides forms iminophosphoranes. In a few reactions phosphazides, the primary imination products have been isolated.[4-6] In some cases iminophosphoranes are trapped via an intramolecular reaction[7] almost in practically quantitative yields. Iminophosphoranes have wide range of applications, such as modification of cell surfaces, protein engineering, specific labeling of nucleic acids, proteomic studies and as a general tool for bioconjugation.[8] Substituted 2-(aminomethy1) piperidines are a novel class of selective protein kinase C [9] which has emerged as a pivotal mediator in cellular regulation, signal transduction and neoplastic promotion. In general, the biological activity of nucleosides is dependent on their ability to be converted intracellularly to the corresponding mono-, di- and tri phosphates by cellular kinases.[10] Some of them are an important class of antiviral and anticancer therapeutics. Nucleoside amino acid phosphoramidate monoesters have been shown to play a potential pronucleotide strategy.[11] In particular, AZT-amino acid phosphoramidates are potent nontoxic antiviral and anticancer agents.[12-16] A major limitation for AZT for the treatment of AIDS is the occurrence of side effect, such as leucopenia. Oxidative stress is involved in AZT-induced leukopenia which may be prevented by treatment with antioxidants.[17] Further

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the relationship between cancer and antioxidants is also a significant factor since there is a battle between cancer and antioxidants. Cancer works against the cells in the body while antioxidants work on behalf of cells.

In view of this back ground, we synthesized iminophosphoranes of zidovudine through Staudinger reaction by incorporating the bioactive groups at the phosphorus atom. They are expected to have better antioxidant activities. The effect of iminophosphoranes of zidovudine on the oxidative stress was evaluated by DPPH, Nitric oxide Scavenging methods. The structures of all the synthesized compounds were established by elemental analyses ,IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data.

METERIALS AND METHODS

Chemicals were procured from Sigma-Aldrich, Merck and Lancaster, and were used as such without further purification. All solvents used for spectroscopic and other physical studies were reagent grade and were further purified by literature methods.[18] Melting points were determined using a calibrated thermometer by Guna Digital Melting Point apparatus. They are expressed in degrees centigrade . IR spectra were obtained on a Perkin-Elmer Model 281-B spectrophotometer in KBr disks and absorptions were reported in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded as solutions in $CDCl_3$ on a Bruker AVANCE III 500 MHz spectrometer operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C and 202.4 MHz for ^{31}P NMR. The ^{1}H, ^{13}C and ^{31}P chemical shifts were expressed in ppm with reference to tetramethylsilane and 85 % H_3PO_4 respectively. Mass spectra were recorded on a JEOL GCMATE II Mass spectrometer. Elemental analyses were performed by Central Drug Research Institute, Lucknow, INDIA.

General Procedure for the Preparation of (4a-l)

A solution of various phenols and amino acid esters (0.002 mole), in dry THF (10 mL) and triethylamine (0.002 mole), was added dropwise over a period of 15 minutes to a stirred solution of phosphorustribromide (0.002 mole) in 10 mL of THF at -15-0 ºC under nitrogen atmosphere. After stirring for 3 hours at 0ºC, formation of intermediate dibromides **2a-l** was ascertained by TLC run in 7:3 mixture of ethyl acetate and hexane. To this (±) 2-amino methyl piperidine (0.002 mole) and triethylamine (0.004 mole), in 10 mL of THF were added dropwise at 0ºC. After completion of the addition, the temperature was slowly raised to RT. The completion of the reaction (3 h) was ascertained by TLC run in 7:3 mixture of ethylacetate and hexane. The reaction mixture was filtered to remove triethylamine hydrobromide. The filtrate containing diazaphospholes (**3a-l**) was taken into a reaction flask under nitrogen atmosphere to which AZT (0.002 mole) in THF (10 mL) was added at RT and slowly raised the temperature to reflux and continued stirring for 2-3 h. The progress of the reaction was monitored by TLC. Solvent was removed in a rotaevaporator and the crude product was purified by column chromatography on silica gel (60-120 mesh) with ethyl acetate: hexane (4:6) as eluent to afford pure iminophosphoranes **4a-l.** All the title compounds were characterized by IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral analyses.

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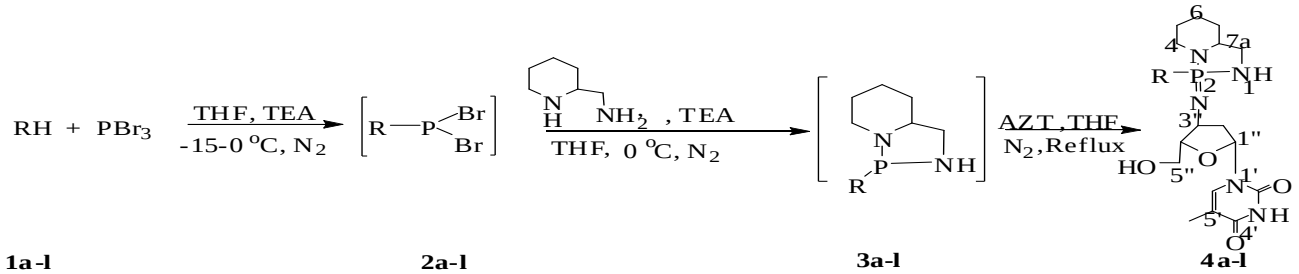
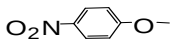
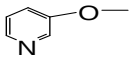
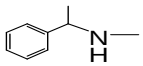
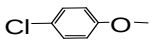
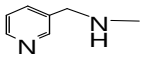
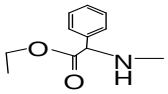
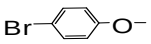
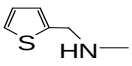
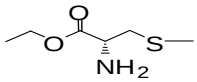
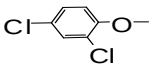
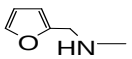
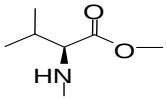


Compound	R	Compound	R	Compound	R
4a		4e		4i	
4b		4f		4j	
4c		4g		4k	
4d		4h		4l	

Scheme-1. Synthesis of 4a-l

1-(5-(hydroxymethyl)-4-{[1-(4-nitrophenoxy)perhydro-1λ^5-[1,3,2]diazaphospholo[1,5a]pyridine-1-yliden] amino}tetrahydro-2- furyl)-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**4a)**: Yield: 65%. M.p: 128-130 ºC. Anal. Calcd. for $C_{22}H_{29}N_6O_7P$: C, 50.77; H, 5.62; N, 16.15. Found C, 50.67; H, 5.59; N, 16.21. IR (KBr): 3496 (-OH), 3369 (-NH), 1731 (C=O), 1258 (P=N), 1213, 948 (P-O-C_{aryl}) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.51 (s, 1H, NH), 8.12 (d, 2H, *J* = 9.5 Hz, Ar-H), 7.53 (d, 2H, *J* = 9 Hz, Ar-H), 7.40 (d, 1H, *J = 1.5* Hz, C<u>H</u>=C), 6.18 (t, 1H, *J* = 13.5 Hz, N-C<u>H</u>-O), 5.35 (br, 1H, P-N<u>H</u>), 4.30-4.34 (m, 1H, CH_2-O<u>H</u>), 4.05-4.1 (m, 3H, C<u>H</u>-CH_2-OH, C<u>H$_2$</u>-OH), 3.65-3.68 (m, 1H, P=N-C<u>H</u>), 2.65-2.71 (m, 2H, NH-C<u>H$_2$</u>), 2.40-2.49 (m, 3H, P-N- C<u>H$_2$</u>, P-N-C<u>H</u>), 2.23-2.37 (m, 2H, P=N-CH-C<u>H$_2$</u>), 1.95 (s, 3H, CH_3), 1.27-1.34 (m, 6H, C<u>H$_2$</u>-C<u>H$_2$</u>-C<u>H$_2$</u>). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 163.7 (C-4$'$), 155.5 (Ar-C-1), 150.2 (C-2$'$), 139.5 (Ar-C-4), 135.6 (C-6$'$), 125.8 (Ar-C-3, Ar-C-5), 121.5 (Ar-C-2, Ar-C-6), 111.5 (C-5$'$), 85.0 (C-4$''$) 82.0 (C-1$''$), 62.2 (C-5$''$), 58.9 (C-7a), 37.2 (C-4), 32.7 (C-8), 31.8 (C-2$''$), 29.6 (C-3$''$), 29.5 (C-7), 29.3 (C-5), 22.6 (C-6), 12.5 (-<u>C</u>H$_3$). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 7.83. GCMS (*m/z*; %): (521.31, 45%) $[MH]^+$.

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1-[4-{[1-(4-bromophenoxy)perhydro-1λ^5-[1,3,2]diazaphospholo[1,5-a]pyridin-1-yliden]amino}-5-(hydroxymethyl)tetrahydro-2-furanyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**4b)**: Yield: 59%. M.p: 135-137 ºC. Anal. Calcd. for $C_{22}H_{29}BrN_5O_5P$: C, 47.66; H, 5.27; N, 12.63. Found C, 47.59; H, 5.18; N, 12.77. IR (KBr): 3487 (-OH), 3355 (-NH), 1729 (C=O), 1254 (P=N), 1215, 947 (P-O-C_{aryl}) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.32 (s, 1H, NH), 7.47 (d, 2H, *J* = 9.5 Hz, Ar-H); 7.44 (d, 2H, *J* = 9.2 Hz, Ar-H), 7.36 (d, 1H, *J* = *1.3* Hz, C<u>H</u>=C), 6.17 (t, 1H, *J* = 12.5 *Hz*, N-C<u>H</u>-O), 5.32 (br, 1H, P-N<u>H</u>), 4.32-4.36 (m, 1H, CH_2-O<u>H</u>), 3.94-3.99 (m, 3H, C<u>H</u>-CH_2-OH, C<u>H$_2$</u>-OH), 3.62-3.65 (m, 1H, P=N-C<u>H</u>), 2.64-2.72 (m, 2H, NH-C<u>H$_2$</u>), 2.43-2.53 (m, 3H, P-N-C<u>H$_2$</u>, P-N-C<u>H</u>), 2.02-2.1 (m, 2H, P=N-CH-C<u>H$_2$</u>), 1.91 (s, 3H, CH_3), 1.25-1.35 (m, 6H, C<u>H$_2$</u>-C<u>H$_2$</u>-C<u>H$_2$</u>). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 163.5 (C-4$^{'}$), 152.1 (Ar-C-1), 150.9 (C-2$^{'}$), 136.8 (C-6$^{'}$), 134.2 (Ar-C-3, Ar-C-5), 123.5 (Ar-C-2, Ar-C-6), 117.5 (Ar-C-4), 110.5 (C-5$^{'}$) 84.9 (C-4$^{''}$) 82.9 (C-1$^{''}$), 61.0 (C-5$^{''}$), 56.9 (C-7a), 36.9 (C-4), 32.9 (C-8), 31.7 (C-2$^{''}$), 29.6 (C-3$^{''}$), 29.6 (C-7), 29.3 (C-5), 22.9 (C-6), 13.1 (-<u>C</u>H$_3$). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 10.4. GCMS (*m/z*; %): (555.30, 30%) $[MH]^+$**.**

1-[4-{[1-(4-chlorophenoxy)perhydro-1λ^5-[1,3,2]diazaphospholo[1,5-a]pyridin-1-yliden]amino}-5-(hydroxymethyl)tetrahydro-2-furanyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**4c)**: Yield 61%. M.p: 127-29 ºC. Anal. Calcd. for $C_{22}H_{29}ClN_5O_5P$: C, 51.82; H, 5.73; N, 13.73. Found C, 51.88; H, 5.79; N, 13.65. IR (KBr): 3491 (-OH), 3364 (-NH), 1730 (C=O), 1262 (P=N), 1213, 948 (P-O-C_{aryl}) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.97 (s, 1H, NH), 7.46 (d, 1H, , *J* = 1.5 Hz, C<u>H</u>=C), 7.37 (d, 2H, *J* = *9.0* Hz, Ar-H); 7.21 (d, 2H, *J* = 8.5 Hz, Ar-H), 6.12 (t, 1H, *J* = 13.5 *Hz*, N-C<u>H</u>-O), 5.34 (br, 1H, P-N<u>H</u>), 4.29-4.34 (m, 1H, CH_2-O<u>H</u>), 4.01-4.06 (m, 3H, C<u>H</u>-CH_2-OH, C<u>H$_2$</u>-OH), 3.68-3.77 (m, 1H, P=N-C<u>H</u>), 2.63-2.68 (m, 2H, NH-C<u>H$_2$</u>), 2.38-2.48 (m, 3H, P-N- C<u>H$_2$</u>, P-N- C<u>H</u>), 2.01-2.09 (m, 2H, P=N-CH-C<u>H$_2$</u>), 1.92 (s, 3H, CH_3), 1.23-1.31 (m, 6H, CH_2-CH_2-CH_2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 163.6 (C-4$^{'}$), 155.3 (Ar-C-1), 150.6 (C-2$^{'}$), 126.1 (Ar-C-3, Ar-C-5), 122.2 (Ar-C-2, Ar-C-6), 140.5 (Ar-C-4), 136.2 (C-6$^{'}$), 111.7 (C-5$^{'}$) 84.9 (C-4$^{''}$) 83.1 (C-1$^{''}$), 61.9 (C-5$^{''}$), 59.2 (C-7a), 37.8 (C-4), 32.8 (C-8), 31.9 (C-2$^{''}$), 29.3 (C-3$^{''}$), 29.5 (C-7), 28.5(C-5), 22.54 (C-6), 12.4 (-<u>C</u>H$_3$), ^{31}P-NMR(202 MHz, $CDCl_3$): δ 6.9 . GCMS (*m/z*; %): (510.23, 30%) $[MH]^+$**.**

1-[4-{[1-(2,4-dichlorophenoxy)perhydro-1λ^5-[1,3,2]diazaphospholo[1,5-a]pyridin-1-yliden] amino}-5-(hydroxymethyl)tetrahydro-2-furanyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidine-dione (**4d)**: Yield 58%. M.p: 132-135 ºC. Anal. Calcd. for $C_{22}H_{28}Cl_2N_5O_5P$: C, 48.54; H, 5.18; N, 12.87. Found C, 48.62; H, 5.21; N, 12.76. IR (KBr): 3489 (-OH), 3359 (-NH), 1729 (C=O), 1264 (P=N), 1218, 950 (P-O-C_{aryl}) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.52 (s, 1H, NH): 7.62 (s, 1H, Ar-H); 7.33 (d, 1H, *J* = 9.5 Hz, Ar-H), 7.29 (d, 1H, *J* = *1.6* Hz, C<u>H</u>=C), 7.09 (d, 1H, *J* = *9.0* Hz, Ar-H), 6.14 (t, 1H, *J* = 12.5 Hz, N-C<u>H</u>-O), 5.31 (br, 1H, P-N<u>H</u>) , 4.30-4.34 (m, 1H, CH_2-O<u>H</u>), 4.07-4.12 (m, 3H, C<u>H</u>-CH_2-OH, C<u>H$_2$</u>-OH), 3.7-3.79 (m, 1H, P=N-C<u>H</u>), 2.63-2.71 (m, 2H, NH-C<u>H$_2$</u>), 2.42-2.5 (m, 3H, P-N- C<u>H$_2$</u>, P-N- C<u>H</u>), 2.01-2.08 (m, 1H, P=N-CH-C<u>H$_2$</u>), 1.93 (s, 3H, CH_3), 1.26-1.36 (m, 6H, C<u>H$_2$</u>-C<u>H$_2$</u>-C<u>H$_2$</u>). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 12.1. GCMS (*m/z*; %): (545.25, 40%) $[MH]^+$**.**

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1-(5-(hydroxymethyl)-4-{[1-(3-pyridyloxy)perhydro-1λ^5-[1,3,2] diazaphospholo[1,5-a] pyridine-1-yliden] amino} tetra hydro-2- furyl)-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**4e)**: Yield 61%. M.p: 141º-143C. IR (KBr): 3492 (-OH), 3365 (-NH), 1725 (C=O), 1258 (P=N), 1210 , 953 (P-O-C_{aryl}) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.63 (s, H, NH), 7.87-7.98 (m, 4H, Ar-H), 7.35 (d, 1H, *J* = 1.4 Hz, CH=C), 6.11 (t, 1H, *J* = 13.5 Hz, N-CH-O), 5.33 (br, 1H, P-NH), 4.31-4.35 (m, 1H, CH2-OH), 4.04-4.1 (m, 3H, CH-CH_2-OH, CH_2-OH), 3.69-3.77 (m, 1H, P=N-CH), 2.65-2.71 (m, 2H, NH-CH_2), 2.39-2.49 (m, 3H, P-N- CH_2, P-N- CH), 2.01-2.09 (m, 1H, P=N-CH-CH_2), 1.92 (s, 3H, CH_3), 1.23-1.31 (m, 6H, CH_2-CH_2-CH_2). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 7.8.

1-[5-(hydroxymethyl)-4-({1-(3-pyridylmethyl)amino]perhydro-1λ^5[1,3,2]diazaphospholo[1,5-a] pyridine-1-yliden}amino)tetra hydro-2- furyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione. (**4f):**Yield 63%. M.p: 153-155 ºC. IR (KBr):. 3497 (-OH), 3369 (-NH), 1725 (C=O), 1248 (P=N) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.68 (s, H, NH), 7.91-8.03 (m, 4H, Ar-H), 7.38 (d, 1H, *J* = 1.5 Hz, CH=C), 6.09 (t, 1H, *J* = 13.0 Hz, N-CH-O), 5.32 (br, 1H, P-NH), 4.28-4.33 (m, 1H, CH_2-OH), 4.15-4.21 (m, 3H, Py-CH_2-NH, Py-CH_2-NH), 3.97-4.03 (m, 3H, CH-CH_2-OH, CH_2-OH), 3.67-3.75 (m, 1H, P=N-CH), 2.64-2.7 (m, 2H, NH-CH_2), 2.37-2.46 (m, 3H, P-N- CH_2, P-N- CH), 2-2.09 (m, 1H, P=N-CH-CH_2), 1.9 (s, 3H, CH_3), 1.23-1.31 (m, 6H, CH_2-CH_2-CH_2). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 11.4.

1-[5-(hydroxymethyl)-4-({1-(2-Thienylmethyl)amino]perhydro-1λ^5[1,3,2]diazaphospholo[1,5-a] pyridine-1-yliden}amino)tetrahydro-2- furyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione. (**4g)**: Yield 64%. M.p:141-143 ºC. Anal. Calcd. for $C_{21}H_{31}N_6O_4PS$: C, 51.00; H, 6.32; N, 16.99. Found C, 51.15; H, 6.39; N, 16.75. IR (KBr): 3490 (-OH), 3362 (-NH), 1730 (C=O), 1245 (P=N) cm^{-1}.^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.7 (s, H, NH), 7.47 (d, 1H, *J* =1.4 Hz, CH=C), 7.37-7.40 (3H, Thio-H), 6.10 (t, 1H, *J* = 13 Hz, N- CH-O), 5.31 (br, 1H, P-NH), 4.39-4.43 (m, IH, CH_2-OH), 4.12-4.19 (m, 3H, Thio-CH_2-NH Thio-CH_2-NH), 4-3.95 (m, 3H, CH_2-OH, CH-CH_2-OH **),** 3.81-3.83 (m, 1H, P=N-CH), 2.49-2.55 (m, 2H, NH-CH_2), 2.38-2.43 (m, 3H, CH_2-N-P, CH-N-P), 2.01-2.09 (m, 1H, P=N-CH-CH_2), 1.24-1.34 (m, 6H, CH_2-CH_2-CH_2), 1.90 (s, 3H, CH_3), 1.24-1.34 (m, 6H, CH_2-CH_2-CH_2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 163.71 (C-4$^{'}$), 150.28 (C-2$^{'}$), 139.51 (Thio-C-2), 136.81 (C-6$^{'}$), 125.9. (Thio-C-3), 126.4 (Thio-C-4), 124.1 (Thio-C-5), 111.25 (C-5$^{'}$), 86.67 (C-4$^{''}$), 84.54 (C-1$^{''}$), 61.95 (C-5$^{''}$), 59.99 (C-7a), 37.31 (C-4), 33.81 (C-8), 31.92 (C-2$^{''}$), 31.42 (Thio-CH_2), 29.69 (C-3$^{''}$), 29.65 (C-7), 29.61 (C-5), 22.68 (C-6), 12.4(-CH_3). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 10.9. GCMS (*m/z*; %): (495.25, 38%) $[MH]^+$**.**

1-[4-({1-[(2-furylmethyl)amino]perhydro-1λ^5-[1,3,2]diazaphospholo[1,5-a]pyridin-1-yliden} amino)-5-(hydroxylmethyl)tetrahydro-2-furanyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidine- dione(**4h):** Yield 59%. M.p:138-141 ºC. IR (KBr): 3489 (-OH), 3365 (-NH),1731 (C=O), 1247 (P=N). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.68 (s, H, NH), 7.45 (d, 1H, *J* = 1.6 Hz, CH=C), 7.39-7.43 (3H, Fu-H), 6.09 (t, 1H, *J* = 12.5 Hz, N- CH-O), 5.29 (br, 1H, P-NH), 4.45-4.49 (m. IH, CH_2-OH), 4.03-3.98 (m, 3H, CH-CH_2-OH, CH_2-OH), 4.1-4.16 (m, 3H, Fu-CH_2-NH, Fu-CH_2-NH), 3.85-3.88 (m, 1H, P=N-CH), 2.36-2.41 (m, 2H, NH-CH_2), 2.25-2.35 (m, 3H, CH_2-N-P, CH-N-P), 2.05-2.12 (m, 1H, P=N-CH-CH_2), 1.87 (s, 3H, CH_3), 1.23-1.34 (m, 6H, CH_2-CH_2-CH_2). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 11.7.

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1-[5-(hydroxymethyl)-4-({1-(1-phenylethyl)amino]perhydro-1λ5[1,3,2]diazaphospholo[1,5-a] pyridine-1-yliden}amino)tetrahydro-2-furyl]-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**4i)**: Yield 63%. M.p: 144-146 ºC. IR (KBr): 3487 (-OH), 3359 (-NH), 1721 (C=O), 1265 (P=N) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.12 (br, H, NH), 7.37 (d, 1H, *J* =1.4 Hz, CH=C), 7.02-7.32 (m, 5H, Ar-H), 6.11 (t, 1H, *J* = 13.5 Hz, N- CH-O), 5.28(s, 1H, P-NH), 4.45-4.52 (m, 2H, Ar-CH-NH Ar-CH-NH) 4.34-4.38 (m. IH, CH_2-OH), 4.03-3.97 (m, 3H, CH-CH_2-OH, CH_2-OH), 3.72-3.67 (m, 1H, P=N-CH), 2.54-2.60 (m, 2H, NH-CH_2), 2.34-2.44 (m, 3H, CH_2-N-P, CH-N-P), 2.04-2.1 (m, 1H, P=N-CH-CH_2), 1.95 (s, 3H, CH_3), 1.64-1.66 (d, 3H, CH_3-CH-Ar), 1.24-1.34 (m, 6H, CH_2-CH_2-CH_2). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 12.31. GCMS (*m/z*; %): (503.79, 20%) $[MH]^+$**.**

Ethyl2-[(1-{[2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetra hydro-3-furanyl]imino}perhydro-1λ^5-[1,3,2]diazaphospholo[1,5-a]pyridin-1-yl)amino]-2-phenyl- acetate (**4j)**: Yield 51% . M.p:147-149 ºC. Anal. Calcd. for $C_{26}H_{37}N_6O_6P$: C, 55.71; H, 6.65; N, 14.99. Found C, 55.68; H, 6.61; N, 15.07. IR (KBr): 3494 (-OH), 3366 (-NH), 1723 (C=O), 1258 (P=N) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.72 (s, H, NH), 7.51-7.60 (5H, Ar-H), 7.40 (d, 1H, *J* = 1.5 Hz, CH=C), 6.07 (t, 1H, *J* = 13.0 Hz, N-CH-O), 5.35 (br, 1H, P-NH), 4.93- 5.01 (m, 2H, Ph-CH, Gly-NH), 4.41-4.45 (m. IH, CH_2-OH), 3.98-4.03 (m, 3H, CH-CH_2-OH,CH_2-OH), 3.82-3.85 (m, 1H, P=N-CH), 3.72 (q, 2H, O-CH_2-CH_3), 2.54-2.60 (m, 2H, NH-CH_2), 2.34-2.44 ((m, 3H, CH_2-N-P, CH-N-P), 2.01-2.09 (m, 1H, P=N-CH-CH_2), 1.94 (s, 3H, CH_3), 1.25-1.35 (m, 6H, CH_2-CH_2-CH_2), 1.12 (t, 3H, J = 6.5 Hz, -CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.2 (Gly-CO), 163.7 (C-4$^{'}$), 155.5 (Ar-C-1), 150.2 (C-2$^{'}$), 125.8 (Ar-C-3, Ar-C-5), 121.5 (Ar-C-2, Ar-C-6), 139.5 (Ar-C-4), 135.8 (C-6$^{'}$), 111.5 (C-5$^{'}$) 85.0 (C-4$^{''}$) 82.0 (C-1$^{''}$), 62.2 (C-5$^{''}$), 58.9 (C-7a), 37.2 (C-4), 32.7 (C-8), 31.8 (C-2$^{''}$), 29.6 (C-3$^{''}$), 29.5 (C-7), 29.5 (C-5), 22.6 (C-6), 12.5(-CH_3). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 12.9. GCMS (*m/z*; %): (561.45, 30%) $[MH]^+$**.**

Ethyl2-amino-3-[(1-{[2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl) tetrahydro-3-furanyl]imino}perhydro-1λ^5-[1,3,2]diazaphospholo[1,5-a]pyridin-1-yl)sulfanyl] propanoate (**4k):** Yield 57%. M.p: 131-133 ºC. IR (KBr): 3488 (-OH), 3359 (-NH), 1725 (C=O), 1258 (P=N) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.61 (s, 1H, NH), 7.38 (d, 1H, *J* = 1.6 Hz, CH=C) 6.06-6.09 (t, 1H, *J* = 12.5 Hz, N- CH-O), 5.31 (br, H, P-NH), 4.52-4.54 (m, 2H, -NH_2,), 4.39-4.44 (m. IH, CH_2-OH), 4.05-3.99 (m, 3H, CH-CH2-OH, CH_2-OH), 3.83-3.79 (m, 2H, P=N-CH, CH-NH_2), 3.69 (q, 2H, -O-CH_2-CH_3), 2.95-2.83 (m, 2H, S-CH_2), 2.52-2.58 (m, 2H, NH-CH_2), 2.4-2.49 (m, 3H, CH_2-N-P, CH-N-P), 1.99-2.06 (m, 1H, P=N-CH-CH_2), 1.89 (s, 3H, CH_3), 1.22-1.33 (m, 6H, CH_2-CH_2-CH_2), 1.15 (t, 3H, O-CH_2-CH_3). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 9.7.

Methyl2-[(1-{[2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetra- hydro-3-furanyl]imino}perhydro-1λ^5-[1,3,2]diazaphospholo[1,5-a]pyridin-1-yl)amino]-3-methylbutanoate (**4l):** Yield 61%. M.p: 126-128 ºC. IR (KBr): 3491 (-OH), 3365 (-NH), 1724 (C=O), 1252 (P=N) cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.59 (s, H, NH), 7.35 (d, 1H, *J* = 1.4 Hz, CH=C), 6.13 (t, 1H, *J* = 13.0 Hz ,N- CH-O), , 5.26 (br, 1H, P-NH), 4.34-4.38 (m, IH, CH_2-OH), 4.2-4.26 (m, H, Val-NH, Val-NH-CH), 4.03-3.97 (m, 3H, CH-CH_2-OH,

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$C\underline{H_2}$-OH), 3.85 (s, 3H, O-CH_3), 3.72-3.76 (m, 1H, P=N-C$\underline{H}$), 2.54-2.60 (m, 2H, NH-$C\underline{H_2}$), 2.34-2.44 (m, 3H, $C\underline{H_2}$-N-P, C$\underline{H}$-N-P), 2.04-2.1 (m, 1H, P=N-CH-$C\underline{H_2}$), 1.94 (s, 3H, CH_3), 1.52-1.46 (m,1H, -C$\underline{H}$-$(CH_3)_2$), 1.25-1.35 (m, 6H, $C\underline{H_2}$-$C\underline{H_2}$-$C\underline{H_2}$), 1.13 (d, 6H, *J* = 6.5 *Hz*, -CH-$(C\underline{H_3})_2$). ^{31}P-NMR (202 MHz, $CDCl_3$): δ 4.4. GCMS (*m/z*; %): (513.1, 58%) $[MH]^+$**.**

ANTIOXIDANT ACTIVITY

The antioxidant activity of the iminophosphorane derivatives of Zidovudine (**3a-l**) has been evaluated and their results are presented in Table 1. The antioxidant activity was evaluated by DPPH,[19] Nitric oxide Scavenging methods.[20] Vitamin C is used as a standard for antioxidant activity.

Table .1

Antioxidant activity of Iminophosphorane derivatives of Zidovudine (4a-l).

Compounds	%DPPH Scavenging activity (µg/mL)	Nitric oxide scavenging Activity (%)
4a	74.45 ± 2.31	65.36 ± 0.72
4b	80.43 ± 1.94	74.15 ± 3.53
4c	78.67 ± 1.23	67 .54 ± 0.21
4d	77.56 ± 1.62	71.56 ± 2.27
4e	73.21 ± 1.64	69.32 ± 1.62
4f	78.82 ±1.26	65.41 ±0.55
4g	79.23 ± 3.01	72 .11 ± 2.18
4h	77.76 ± 1.81	70.54 ± 1.98
4i	79.19 ± 2.43	72.19 ± 2.22
4j	81.61 ± 1.25	76.43 ± 1.54
4k	79.73 ± 2.12	73.73 ± 2.12
4l	78 .43± 2.89	72.38± 1.09

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AZT	70.34 ± 1.32	67.27 ± 1.36
Vitamin C	82.30 ± 1.66	79.65± 1.23

DPPH free radical-scavenging assay

The antiradical activity of iminophosphoranes of AZT (**4a-l**) were measured in triplicate using a modified Yamaguchi *et al.* method. A methanolic solution of DPPH (4 mL, 40 ppm) was added to 1 mL of the antioxidant solutions in 0.1 M Tris–HCl buffer (pH 7.1) at 25 ºC, giving final concentrations of 10, 25, 50 and 100 µM. The absorbance read at 517 nm was measured after 60 min of the reaction in the dark and compared with the control prepared in a similar way without the addition of the test compounds. A 100 µM solution of Vitamin C (a strong and natural antioxidant agent) was also tested as a control for the reaction. In this case, the violet color of DPPH disappeared immediately. We confirmed that the test compounds (**4a-l**) showed significant antioxidant activity when compared to the parent drug and Vitamin C.

$$\text{Antioxidant activity} = [(A_{517}\text{control} - A_{517}\text{sample}) / A_{517}\text{control}] \times 100$$

Nitric oxide scavenging activity

.Sodium nitroprusside (5 mmol L^{-1}) in phosphate buffered saline pH 7.4, was mixed with the test sample prepared in methanol and incubated at 25 °C for 30 min. A control without the test compound, but with an equivalent amount of methanol, was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% *N*-1-naphthylethylenediamine dihydrochloride). UV-absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with *N*-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and the per centage scavenging activity was measured with reference to the standard.

RESULTS AND DISCUSSION

All the title compounds **4a-l** exhibited characteristic infrared absorption bands for P=N, C=O, -NH and -OH in the regions 1245-1265, 1723-1731 3355-3369 and 3487-3497 cm^{-1}.[21] The NH protons in thymine gave singlets in the region δ 8.97-9.7.[22, 23] The both NH, NH-CH protons of amino acid ester moieties appeared as a multiplet.[24] The P–NH proton resonated as a broad singlet in the region 5.26-5.35 ppm. The H-6' proton signal appeared as a doublet in the region 7.47-7.29 ppm.[25] The ^{13}C NMR spectral data for **4a**, **4b**, **4c**, **4g**, **4j** are given in the experimental section. The chemical shifts of the carbonyl carbon atom of thymine ring[25] appeared at δ 163.8-150.2. The data of other carbon signals are observed in the expected region. The ^{31}P NMR chemical shifts appeared in the down field region 6.9-12.9 ppm as expected,[1] due to the deshielding effect of nitrogens and oxygen atoms around P=N system. GC Mass spectra were recorded for a few compounds and they exhibited molecular ion peaks with moderate intensity.

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The Iminophosphorane derivatives (**4a-l**) exhibited more antioxidant activity when compared to parent compound AZT. Among **4a-l**, **4b, 4j** and **4k** exhibited very promising antioxidant activity, **4j** exhibited very significant DPPH radical scavenging activity when compared to Vitamin C.

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