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Der Pharma Chemica, 2010, 2(4): 1-9
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Synthesis and antimicrobial activity evaluation of novel phosphorylated derivatives of zidovudine

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Abstract

*A series of novel phosphorylated derivatives of zidovudine (**5-15**) were synthesized in two step process with high yields. In the first step zidovudine (**1**) was reacted with bis(2-chloroethyl) phosphoramidicdichloride/4-nitrophenylphosphorodichloridate/4-chloro phenyl phosphorodi-chloridate in presence of triethylamine (TEA) in dry tetrahydrofuran (THF) to yield the intermediates **(2-4)**. They were further reacted with various compounds like monopotassium dihydrogenphosphate and amino acid esters to obtain the title compounds **5-15**. The title compounds showed promising antimicrobial activity and it was greatly influenced by the presence of different bio-active groups at phosphorus.*

Key words: phosphorylated derivatives of zidovudine, gram positive bacteria, gram negative bacteria, antimicrobial activity, phosphoramidate monoesters

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INTRODUCTION

Nucleosides are an important class of antiviral and anticancer therapeutics. 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 [1]. Phosphate esters substituted with amino acid esters at phosphorus are important class of rationally designated therapeutics especially with antineoplastic properties [2]. Phosphate triester derivatives of nucleotides have been prepared as the membrane-soluble prodrug of the bioactive nucleotides and were evaluated against HIV-1 *in vitro* [3]. The aryloxy substituted phospharamidates were found to exhibit enhanced activity against HIV-1 and HIV-2 in cell culture compared to their parent ddN's with full retention of activity in thymidine kinase deficient cell lines [4-6]. This type of nucleotides has been shown to be potent inhibitors of HIV and to display reduced toxicity in certain cell types [7-9]. Exhaustive modifications to the amino acid moiety in aryloxy phosphoramidate have established L-alanine as the moiety for optimal antiviral activity [10]. Some of the phosphorus compounds belonging to nucleotide family such as AZT 5′-phosphate

and its isosteric phosphonate [11, 12] are also reported to be good antiviral agents. The hydrolysis of the amino acid ester moiety of these compounds during metabolism release products of limited toxicity into the system [13]. In view of this we have accomplished synthesis of a new class of phosphorylated derivatives of zidovudine and evaluated their antimicrobial activity.

MATERIALS AND METHODS

Experimental

Chemicals were obtained from Sigma-Aldrich, used as such without further purification. All solvents (AR grade) used for spectroscopic and other physical studies were further purified by literature methods. All operations were performed under nitrogen atmosphere using standard glass wares. Melting points were determined using a calibrated thermometer by Guna Digital Melting Point apparatus. Elemental analyses were performed by University of Hyderabad, Hyderabad. IR spectra were recorded in Environmental Engineering Laboratory, Sri Venkateswara University, Tirupati as KBr discs on a Nicolet-380 FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded as solutions in DMSO-d_6 on a Bruker AMX 400 MHz spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 161.9 MHz for ^{31}P. The ^{1}H and ^{13}C chemical shifts were referenced to tetramethylsilane (TMS) and ^{31}P chemical shifts to 85 % H_3PO_4. APCI mass spectra were recorded on a Jeol SX 102 DA / 600 mass spectrometer.

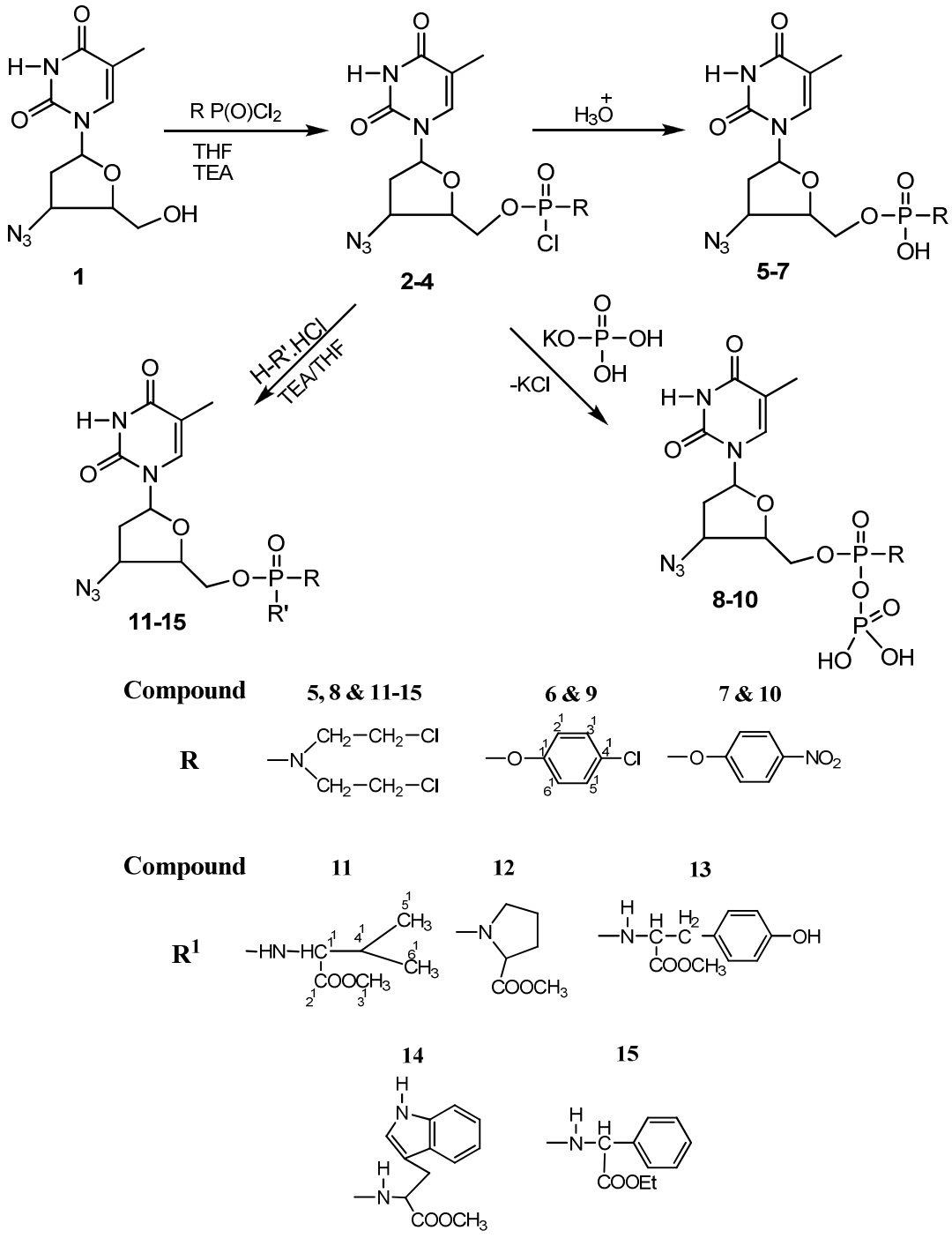
RESULTS AND DISCUSSION

The chemical structures of all the title compounds **5-15** were characterized by IR, ^{1}H, ^{13}C, ^{31}P-NMR and APCI-MS studies and their data are presented in the experimental section. To a cooled solution of Zidovudine **1** in 20 mL of THF and TEA, alkyl/aryl phosphorodichloridates were added slowly to get the corresponding monochlorides **2-4,** then they were hydrolyzed to get compounds **5-7**. **2-4** were treated with monopotassium hydrogen phosphate to get **8-10** and also reacted with various amino acid esters to obtain **11-15**. All the compounds **5-15** showed characteristic infrared absorption bands for OH, NH, C=O and P=O in the region 3418-3479, 3380-3390, 1650-1668 and 1234-1259 cm^{-1} respectively. The IR spectral data confirm [14] the functional groups present in the compounds **5-15**. The NH protons in thymidine ring were observed [15,16] as a singlet in the region δ 10.03-10.21. The aromatic protons of the titled compounds showed complex multiplets [16,17] in the region δ 6.40 to7.82. The remaining protons of amino acid esters were observed in the expected region [14]. The ^{13}C-NMR chemical shifts for compounds **5-15** were observed at expected region [16-19]. The ^{31}P-NMR chemical shifts of **5-15** appeared in the region -2.01 to 2.13 ppm as expected [16-19].

Pharmacology

Antibacterial Activity

Antibacterial activity of all the title compounds (**5-15**) was assayed [20] 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 1). Solvent control was included although no antibacterial activity has been noted in the solvent employed. Penicillin-G (Hi-media) controls (20 μg/mL) were included to compare with compounds **5-15**. All samples were tested in triplicate and average results were recorded.



Scheme –1

Disc diffusion bioassay: For the bioassay a suspension of approximately 1.5 × 108 bacterial cells per mL was used. 1.5 mL of the bacterial suspension was uniformly spread on nutrientdisc was introduced onto the upper layer of the medium seeded with bacteria. The Petri dishes were incubated at 35 °C for 24 h. Bioactivity was determined by measuring the diameters of the inhibition zones in mm. The compounds **5-15** were made up at 25, 50 and 100 µg mL^{-1} concentrations for bio-activity screening by the disc method. Each test was done in triplicate and the mean diameter of the inhibition zones was calculated. Controls included the use of solvent without test compounds although no antibacterial activity was noted for the solvent employed in the test. The minimum inhibitory concentration (MIC) was determined for the compounds 3a–l used at concentrations of 0.1–5.6 mg mL–1. Specifically 0.1 mL of standardized inoculum (1–2

× 107CFUmL–1) was added to each tube. The tubes were incubated aerobically at 35 °C for 24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing the growth medium without inoculum) and organism control (tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the compounds **5-15** that produced no visible bacterial growth (no turbidity)whencompared with the control tubes was regarded as MIC. The highlight is that the majority of compounds exhibited high activity against both bacteria and two compounds (3e and 3f) were more effective than the standard compound. Penicillin was tested as a standard reference to compare the activities of these compounds.

Table 1: Antibacterial activity of title compounds 5-15

Compd.	Zone of inhibition (mm)					
	Staphylococcus aureus			*Escherichia coli*		
	100 ppm[a]	50 ppm[a]	25 ppm[a]	100 ppm[a]	50 ppm[a]	25 ppm[a]
5	11	8	6	12	8	4
6	8	6	-	10	7	5
7	10	8	6	12	8	4
8	6	5	4	12	6	6
9	14	9	5	14	12	8
10	13	11	8	13	11	7
11	7	4	-	9	8	4
12	10	8	5	10	6	5
13	12	10	8	10	6	4
14	11	8	5	12	8	6
15	10	8	4	10	7	5
[b]*Penicillin G*	9	6	-	12	8	-

[a] In DMF; [b] Reference Compound.

Antifungal Activity

The compounds **5-15** were screened for their antifungal activity (Table 2) against *Aspergillus niger* (ATCC 16404) and *Helminthosporium oryzae* (ATCC 11000) species along with standard fungicide Griseofulvin at three different concentrations (100, 50 and 25 ppm) in DMF [21]. All the compounds **5-15** exhibited moderate to high antifungal activity when compared with that of the reference compound. The majority of the compounds exhibited high activity against fungi.

1-[2-([Di(2-chloroethyl)amino]hydroxyphosphoryloxy)methyl]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (5).

To a cooled (10 ºC) and stirred solution of Zidovudine **1** (0.33 g, 0.0012 mol) in 20 mL of dry THF and TEA (0.12 g, 0.0012 mol), a solution of bis (2-chloroethyl) phosphoramidic dichloride (0.32 g, 0.0012 mol) in 15 mL of dry THF was added drop wise over a period of 20 min. After completion of addition, the temperature of the reaction mixture was raised to room temperature and stirred for 1 h to form the intermediate **2,** and $Et_3N.HCl$ was separated by filtration, then added few drops of distilled water in TEA slowly. The progress of the reaction was judged by TLC analysis (ethyl acetate: hexane 1:2). After completion of the reaction the solvent was removed in a rota-evaporator, the resulting crude product was recrystallized from 2-

propanol to obtain pure compound of **5**. Yield (0.40 g, 70 %), mp 181-183 ºC. The same procedure was adopted for the preparation of compounds **6** and **7**.

Table 2: Antifungal activity[a] of title compounds 5-15

Compd.	Zone of inhibition/mm					
	Aspergillus niger			*Helminthosporium oryzae*		
	100 ppm[a]	50 ppm[a]	25 ppm[a]	100 ppm[a]	50 ppm[a]	25 ppm[a]
5	10	7	5	11	6	5
6	11	8	4	11	9	5
7	12	9	6	12	10	7
8	13	10	8	14	10	4
9	10	7	5	12	8	7
10	9	5	3	12	10	9
11	10	6	4	9	8	4
12	9	8	6	11	9	5
13	14	11	9	13	12	8
14	8	9	6	9	7	4
15	8	6	5	10	10	3
[b]*Griseofulvin*	12	10	5	12	10	5

[a]In DMF; [b]Reference Compound

1-[2-([Di(2-chloroethyl)amino](phosphonooxy)phosphoryl]oxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (6).
To a cooled (10 ºC) and stirred solution of Zidovudine **1** (0.33 g, 0.0012 mol) in 20 mL of dry THF and TEA (0.12 g, 0.0012 mol), a solution of bis (2-chloroethyl) phosphoramidic dichloride (0.32 g, 0.0012 mol) was added drop- wise over a period of 20 min. After completion of addition, the temperature of the reaction mixture was raised to room temperature and stirred for 1 h to form the intermediate **2,** and $Et_3N.HCl$ was separated by filtration then added monopotassium di hydrogenphosphate (0.17 g, 0.0012 mol) and stirred for another 1 h. The progress of the reaction was judged by TLC analysis (ethyl acetate: hexane 1:1). Potassium bromide salt was separated by filtration. The solvent was removed in a rota-evaporator, the resulting crude product was recrystallized from 2-propanol to obtain pure compound of **8**. Yield (0.48 g, 72 %), mp 187-189 ºC. The same procedure was adopted for the preparation of compounds **9** and **10**.

1-[2-([Di(2-chloroethyl)amino][1-methoxycarbonyl)-2-methylpropyl]amino-phosphoryl) oxy]methyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (11).
To a cooled (10 ºC) and stirred solution of Zidovudine (**1**) (0.33 g, 0.0012 mol) in 20 mL of dry THF and TEA (0.12 g, 0.0012 mol), a solution of bis (2-chloroethyl) phosphoramidic dichloride (0.32 g, 0.0012 mol) in 15 mL of dry THF was added drop-wise over a period of 20 min. After completion of addition, the temperature of the reaction mixture was raised to room temperature and stirred for 1 h to form the intermediate **2**. Then a solution of L-valinemethyl ester HCl (0.16 g, 0.0012 mol) in 30 mL of dry THF was added slowly and then stirred for another 1 h. The progress of the reaction was judged by TLC analysis (ethyl acetate: hexane-1:1). After

completion of the reaction, $Et_3N.HCl$ was separated by filtration and the solvent was removed in a rota-evaporator the resulting crude product was recrystallized from 2-propanol to obtain pure compound of **11**. Yield (0.47 g, 65 %), mp 221-223 ºC. The same procedure was adopted for the preparation of compounds **12-15**.

1-[2-([Di(2-chloroethyl)amino]hydroxyphosphoryloxy)methyl]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (5):
Yield: 70 %; mp: 181-183 ^{0}C; IR (KBr): 3425 (O-H), 3396 (N-H), 1259 (P=O); cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.03 (s, 1H, NH), 7.43 (s, 1H, CH=C), 6.27 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.39-4.51 (m, 1H, CH-N_3), 4.18-4.21 (m, 2H, O-CH_2), 4.05-4.09 (m, 1H, CH-CH_2), 2.22-2.27 (m, 2H, CH-CH_2), 2.81 (s,1H, OH), 3.49 (t, *J* = 7.5 Hz, 4H, N-CH_2), 2.74 (t, *J* = 7.5 Hz, 4H CH_2-Cl,), 1.84 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 151.2 (C-1), 164.3 (C-2), 120.5 (C-3), 22.5 (C-4), 142.0 (C-5), 89.1 (C-6), 40.5 (C-7), 41.6 (C-8), 85.8 (C-9), 66.3 (C-10), 49.4 (C-11 &13), 41.6 (C-12 &14); ^{31}P-NMR (85 % H_3PO_4): δ 1.02; APCIMS: m/z (%): 47 ($M^{+\bullet}$, 58), 438 (35), 337 (20), 319 (40), 239 (31), 218 (100), 118 (8); Anal. Calcd (%) for $C_{14}H_{21}Cl_2N_6O_6P$: C, 35.68; H, 4.49; N, 17.83; Found: C, 35.66; H, 4.46; N, 17.81.

1-[2-([(4-Chlorophenoxy)]hydroxyphosphoryloxy)methyl]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro - 1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (6):
Yield 72 %; mp: 162-163 ^{0}C; IR(KBr): 3427 (O-H), 3383 (N-H), 1252 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.04 (s, 1H, NH), 7.41 (s, 1H, CH=C), 7.16 (d, *J*=8.2 Hz, 2H, ArH), 6.78 (d, *J* = 8.2 Hz, 2H, ArH) 6.27 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.39-4.41 (m, 1H, CH-N_3), 4.20-4.21 (m, 2H, O-CH_2), 4.04-4.07 (m, 1H, CH-CH_2), 2.22 (m, 2H, CH-CH_2), 2.91 (s, 1H, OH), 1.83 (s, 3H, CH_3); ^{31}P-NMR (85 % H_3PO_4): δ 1.12; Anal. Calcd (%) for $C_{16}H_{17}ClN_5O_7P$: C, 41.98; H, 3.74; N, 15.30; Found: C, 41.95; H, 3.72; N, 15.27.

1-[2-([(4-Nitrophenoxy)]hydroxyphosphoryloxy)methyl]-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro -1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (7):
Yield 68 %; mp: 168-169 ^{0}C; IR(KBr): 3429 (O-H), 3389 (N-H), 1238 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, NH), 8.02 (d, *J* = 8.2 Hz, 2H, ArH), 7.44 (s, 1H, CH=C), 7.02 (d, *J* = 8.2 Hz, 2H,ArH),6.28 (t,*J*=7.5Hz,1H,N-CH-O),4.39-4.43 (m, 1H, CH-N_3), 4.17-4.21 (m, 2H, O-CH_2), 4.03-4.08 (m, 1H, CH-CH_2), 2.22-2.26 (m, 2H, CH-CH_2), 2.91 (s, 1H, OH), 1.85 (s, 3H, CH_3); ^{31}P-NMR (85 % H_3PO_4): δ 1.19; APCIMS: m/z (%): 468 ($M^{+\bullet}$, 23), 413 (48), 384 (39), 291 (12), 266 (100), 216 (26), 186 (66), 126 (17); Anal. Calcd (%) for $C_{16}H_{17}N_6O_9P$: C, 41.04; H, 3.66; N, 17.94; Found: C, 41.01; H, 3.62; N, 17.91.

1-[2-([Di(2-chloroethyl)amino](phosphonooxy)phosphoryl]oxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (8):
Yield 72 %; mp: 187-189 ^{0}C; IR(KBr): 3425 (O-H), 3384 (N-H), 1234 ^{1} (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.03 (s, 1H, NH), 7.41 (s, 1H, CH=C), 6.27 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.39-4.42 (m, 1H, CH-N_3), 4.17-4.22(m,2H,O-CH_2),4.07-4.10(m, 1H, CH-CH_2), 2.24-2.28 (m, 2H, CH-CH_2), 2.87 (s,1H, OH), 3.50 (t, *J* = 7.5 Hz, 4H, N-CH_2), 2.72 (t, *J* = 7.5 Hz, 4H, CH_2-Cl), 2.19 (s, 1H, OH), 1.83 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 154.3 (C-1), 168.2 (C-2), 116.3 (C-3), 19.3 (C-4), 140.4 (C-5), 88.4 (C-6), 40.4 (C-7), 42.4 (C-8), 84.1 (C-9), 65.5 (C-10), 49.3 (C-11 & 13), 41.3 (C-12 & 14); ^{31}P-NMR (85 % H_3PO_4): δ -0.42 and 1.02; APCIMS: m/z (%): 550 ($M^{+\bullet}$, 54), 487 (18), 367 (30), 267 (100), 186 (38), 156 (89), 129 (60), 92 (20); Anal. Calcd (%) for $C_{14}H_{22}Cl_2N_6O_9P_2$: C, 30.51; H, 4.02; N, 15.25; Found: C, 30.48; H, 4.00; N, 15.21.

1-[2-([(4-Chlorophenoxy)(phosphonooxy)phosphoryl]oxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (9):
Yield 71 %; mp: 175-176 ^{0}C; IR(KBr): 3422 (O-H), 3385 (N-H), 1238 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ10.02 (s,1H, NH), 7.43 (s,1H, CH=C), 7.17 (d, *J* = 8.2 Hz, 2H, ArH), 6.74 (d, *J* = 8.2 Hz, 2H, ArH), 6.26 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.40-4.43 (m, 1H, CH-N$_3$), 4.16-4.19 (m, 2H, O-CH$_2$), 4.04-4.07 (m, 1H, CH-CH$_2$), 2.20-2.23 (m, 2H, CH-CH$_2$), 2.91 (s, 2H, OH), 1.83 (s, 3H, CH$_3$); ^{31}P-NMR (85 % H_3PO_4): δ –0.42 and 1.02; Anal. Calcd (%) for $C_{16}H_{18}ClN_5O_{10}P_2$: C, 35.74; H, 3.37; N, 13.02; Found: C, 35.70; H, 3.33; N, 12.98.

1-[2-([(4-Nitrophenoxy)(phosphonooxy)phosphoryl]oxymethyl)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (10):
Yield 69 %; mp: 178-179 ^{0}C; IR(KBr): 3427 (O-H), 3388 (N-H), 1252 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, NH), 8.04 (d, *J* = 8.2 Hz, 2H, ArH), 7.44 (s, 1H, CH=C), 7.04 (d, *J* = 8.2 Hz, 2H, ArH), 6.28 (t, *J*=7.5 Hz,1H, N-CH-O), 4.39 -4.43 (m, 1H, CH-N$_3$), 4.20-4.24 (m, 2H, O-CH$_2$), 4.05-4.07 (m, 1H, CH-CH$_2$), 2.22-2.25 (m, 2H, CH-CH$_2$), 2.61 (s,1H, OH), 1.85 (s, 3H, CH$_3$); ^{31}P-NMR (85 % H_3PO_4): δ –0.48 and 1.21; APCIMS (%):548 ($M^{+\bullet}$, 53), 533 (41), 512 (34), 484 (15), 323 (26), 266 (100), 221 (30), 188 (40), 176 (20), 105 (26.

1-[2-([Di(2-chloroethyl)amino][1-methoxycarbonyl)-2-methylpropyl]amino-phosphoryl)oxy]methyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (11):
Yield 65 %; mp: 221-223 ^{0}C; IR (KBr): 3384 (N-H), 1678 (C=O),1242 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ10.04 (s,1H, NH), 7.43 (s, 1H, CH=C), 6.28 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.36-4.39 (m, 1H, CH-N$_3$), 4.19-4.22 (m, 2H, O-CH$_2$), 4.06-4.09 (m, 1H, CH-CH$_2$), 2.22-2.25 (m, 2H, CH-CH$_2$), 1.85 (s, 3H, CH$_3$), 4.71 (d, *J* = 8.2Hz, 1H, NH), 2.30 (d, *J* =10.2 Hz,1H, CH-CO), 3.69 (s, 3H, O-CH$_3$), 2.10-2.12 (m, 1H, CH(CH$_3$)$_2$), 1.16 (d, *J*=8.6Hz, 6H, CH (CH$_3$)$_2$), 3.50 (t, *J*=7.5 Hz, 4H, N-CH$_2$), 2.75 (t, *J* = 7.5 Hz, 4H, CH$_2$-Cl); ^{13}C-NMR (DMSO-d_6): δ 154.3 (C-1), 168.2 (C-2), 116.3 (C-3), 19.3 (C-4), 140.4 (C-5), 88.4 (C-6), 40.4 (C-7), 42.4 (C-8), 85.2 (C-9), 67.1 (C-10), 50.3 (C-11 & 13), 42.2 (C-12 & 14), 54.1 (C-1^1), 175.3 (C-2^1), 53.2 (C-3^1), 35.3 (C-4^1), 20.1 (C-5^1& 6^1); ^{31}P-NMR (85 % H_3PO_4): δ 2.11 ppm; APCIMS (%): 583($M^{+\bullet}$, 33), 498 (38), 452 (26), 384 (66), 266 (100), 232 (45), 202 (24), 186 (30), 106 (23); Anal. Calcd (%) for $C_{20}H_{32}Cl_2N_7O_7P$: C, 41.11; H, 5.52; N, 16.78: Found: C, 41.09; H, 5.50; N, 16.75.

1-[2-([Di(2-chloroethyl)amino][1-methoxycarbonyl)-2-pyrrolidinephosphoryl) oxy]methyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1,2-triazadien-2-ium (12):
Yield 68 %; mp: 217-219 ^{0}C; IR(KBr): 3385 (N-H), 1666 (C=O), 1252 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): (δ ppm) 10.02 (s, 1H, NH), 7.41 (s, 1H, CH=C), 6.29 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.40-4.43 (m, 1H, CH-N$_3$), 4.19-4.21 (m, 2H, O-CH$_2$), 4.07-4.10 (m, 1H, CH-CH$_2$), 2.23-2.26 (m, 2H, CH-CH$_2$), 1.83 (s, 3H, CH$_3$), 3.51 (t, *J* = 7.5 Hz, 4H, N-CH$_2$), 2.72 (t, *J* = 7.5 Hz, 4H, CH$_2$-Cl), 3.68 (s, 3H, O-CH$_3$), 3.13-4.24 (m, 1H, CH), 2.07 (t, 2H, CH$_2$), 1.91-2.02 (m, 2H, CH$_2$), 1.64-1.79 (m, 2H, CH$_2$); ^{31}P-NMR (85 % H_3PO_4): δ 2.13 ppm; APCIMS (%): 58 ($M^{+\bullet}$, 40), 566 (37), 512 (40), 492 (22), 423 (27), 376 (19), 267 (100), 251 (33), 198 (42), 155 (23), 140 (44); Anal. Calcd (%) for $C_{20}H_{30}Cl_2N_7O_7P$: C, 41.25; H, 5.19; N, 16.84: Found: C, 41.20; H, 5.16; N, 16.80.

1-[2-([Di(2-chloroethyl)amino][1-(4-hydroxybenzyl)-2-methoxy-2-oxoethyl] aminophosphoryl)oxy]methyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl) tetrahydro-3-furanyl]-1,2-triazadien-2-ium (13):

Yield 64 %; mp: 243-245 ^{0}C; IR (KBr): 3424 (O-H), 3388 (N-H), 1672 (C=O),1234 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ10.02 (s,1H, NH), 7.44 (s,1H, CH=C), 6.26 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.41-4.44 (m, 1H, CH-N$_3$), 4.17-4.20 (m, 2H, O-CH$_2$), 4.07-4.10 (m, 1H, CH-CH$_2$), 2.25-2.28 (m, 2H, CH-CH$_2$), 1.86 (s, 3H, CH$_3$), 3.50 (t, *J* = 7.5 Hz, 4H, CH$_2$Cl), 2.76 (t, *J* = 7.5 Hz, 4H, NCH$_2$), 4.53 (t, *J* = 7.5 Hz, 1H,-NH-CH), 2.67 (q,1H,-CH-C=O),2.37 (d, *J*=10.2 Hz, 2H,-CH-CH$_2$-C=C), 3.68, 3H, O-CH$_3$), 11.1 (s, 1H, Ar-OH), 6.71-6.98 (m, 4H, Ar-H); ^{31}P-NMR (85 % H_3PO_4): δ 1.97 ppm; Anal. Calcd (%) for $C_{24}H_{32}Cl_2N_7O_8P$: C, 47.07; H, 5.62; N, 12.67: Found: C, 47.03; H, 5.60; N, 12.63.

1-[2-([Di(2-chloroethyl)amino][1-(1*H*-3-indolylmethyl)-2-oxoethyl]amino-phosphoryl) oxy]methyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl) tetrahydro-3-furanyl]-1,2-triazadien-2-ium (14):

Yield 61 %; mp: 256-258 ^{0}C;IR (KBr): 3387 (N-H), 1672 (C=O),1235 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ10.03 (s, 1H, NH), 7.45 (s, 1H, CH=C), 6.25 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.36-7.39 (m, 1H, CH-N$_3$), 4.15-4.19 (m, 2H, O-CH$_2$), 4.07-4.11 (m, 1H, CH-CH$_2$), 2.24-2.27 (m, 2H, CH-CH$_2$), 1.85 (s, 3H, CH$_3$), 3.51 (t, *J* = 8.5 Hz, 4H, CH$_2$Cl), 2.76 (t, *J* = 7.5 Hz, 4H, NCH$_2$), 4.67 (t, *J* = 8.2 Hz, 1H, -NH-CH-CH$_2$), 2.68 (q, 1H, -NH-CH-CH$_2$), 2.47 (d, *J*=11.8 Hz, 2H, -NH-CH-CH$_2$), 3.68 (s, 3H, O-CH$_3$), 10.91 (s, 1H, NH-Ar), 6.67-7.19 (m, 5H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ 154.2 (C-1), 168.2 (C-2), 116.4 (C-3), 19.3 (C-4), 140.4 (C-5), 88.2 (C-6), 40.4 (C-7), 42.4 (C-8), 85.5 (C-9), 68.3 (C-10), 51.3 (C-12 & 14), 42.4 (C-11 & 13), 54.1 (C-1^1), 174.1 (C-2^1), 52.2 (C-3^1), 34.3 (C-4^1), 113.1 (C-5^1), 131.3 (C-6^1), 121.2 (C-7^1), 125.1 (C-8^1), 122.2 (C-9^1), 113.5 (C-10^1), 141.3 (C-11^1), 125.5 (C-12^1); ^{31}P-NMR (85 % H_3PO_4): δ 1.20 ppm; APCIMS (%):670 ($M^{+\bullet}$, 21), 579 (40), 532 (36), 498 (28), 421 (17), 396 (23), 267 (100), 258 (10), 208 (22), 198 (56), 115 (61); Anal. Calcd (%) for $C_{26}H_{33}Cl_2N_8O_7P$: C, 46.51; H, 4.95; N, 16.69: Found: C, 46.48; H, 4.92; N, 16.66.

1-[2-([Di(2-chloroethyl)amino][1-ethoxy-2-oxo-1-phenylethyl)amino]phosphoryloxy] methyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl) tetrahydro-3-furanyl]-1,2-triazadien-2-ium (15):

Yield 70 %; mp: 243-245 ^{0}C ;IR (KBr): 3389 (N-H), 1674 (C=O), 1237 (P=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.03 (s,1H, NH), 7.43 (s,1H, CH=C), 6.27 (t, *J* = 7.5 Hz, 1H, N-CH-O), 4.39-4.12 (m, 1H, CH-N$_3$), 4.18-4.21 (m, 2H, O-CH$_2$), 4.05-4.09 (m, 1H, CH-CH$_2$), 2.23-2.26 (m, 2H, CH-CH$_2$), 1.84 (s, 3H, CH$_3$), 3.49 (t, *J* = 7.5 Hz, 4H, N-CH$_2$), 2.74 (t, *J* = 7.5 Hz, 4H, CH$_2$-Cl), 4.77 (t, *J* = 7.2 Hz, 1H, NH), 2.29 (d, *J* = 10.2 Hz, 1H, CH-CO), 3.61 (q, 2H, O-CH$_2$-CH$_3$), 1.19 (t, *J*=7.5 Hz, 3H, O-CH$_2$-CH$_3$), 6.72-7.03 (m, 5H, Ar-H); ^{31}P-NMR (85 % H_3PO_4): δ 1.17; APCIMS (%): 532 ($M^{+\bullet}$,35) 515 (40), 491 (18), 420 (29), 396 (39), 267 (100), 251 (32), 198 (40), 155 (21), 140 (19); Anal. Calcd (%) for $C_{24}H_{32}Cl_2N_7O_7P$: C, 46.00: H, 5.26; N, 17.03: Found: C, 45.96: H, 5.24; N, 17.05.

CONCLUSION

In summary, a series of novel phosphorylated derivatives of zidovudine were synthesized and evaluated their antimicrobial activity. Structure-activity studies showed that the antimicrobial potency was mainly influenced by the functional groups at the end of the aliphatic chain, as well as the nature of atom attached directly to phosphorus atom and the three different types of R fragments attached to phosphorus atom, which provided additional sites of interactions between the inhibitor and the enzyme, which constitutes a key element for enhanced affinity.

Acknowledgments
The authors thankful BRNS (DAE), BARC, Mumbai, India, for providing financial assistance through the project (2007/37/46/BRNS/2916, dated 31-03-2008) and Dr. P. Chandra Mohan Reddy, Department of Microbiology, Yogi Vemana University, Kadapa, India for conducting anti-microbial activity

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