

Nucleoside substituted perhydro-1 λ 5-[1,3,2]diazaphospholo[1,5-a]pyridine-1-thione analogues: Synthesis and evaluation of antiviral and antimicrobial activities

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Abstract: A new series of nucleoside substituted perhydro-1 λ 5-[1,3,2]diazaphospholo[1,5-a]pyridine-3-thione derivatives **4(a-j)** was synthesized by a one-pot two-step process. It involves the formation of key intermediate, 1-chloroperhydro-1 λ 5-[1,3,2]diazaphospholo [1,5-a]pyridine-1-thione (**3**) and its subsequent reaction with various nucleosides to obtain the title products **4(a-j)**. Structures of all the newly synthesized compounds were elucidated by spectral analysis. Compound (**4b**) linked with zidovudine exhibited highest antiviral and antimicrobial activities as compared to other nucleoside derivatives.

Keywords: 2(\pm)-Aminomethyl piperidine; thiophosphoryl chloride; nucleosides; antiviral activity; antimicrobial activity. ©2018 ACG Publication. All right reserved.

1. Introduction

Organophosphorus compounds are interesting frame works since they find in various applications such as pharmaceuticals, agrochemicals and chemical synthesis.¹⁻³ In particular, phosphorus heterocyclic compounds with five and six membered consisting of heteroatoms like nitrogen and oxygen have been attained significant interest due to their important applications in organic synthesis⁴ and large varieties of biological activities such as herbicidal,^{4,5} insecticidal⁶ and antimicrobial.⁷ Further, the phosphorous compounds with P-N functionality like diazaphospholes have been attained great interest due to their significant medicinal, pesticidal,⁸⁻¹¹ antiviral, bactericidal, antioxidant,¹² and anti-carcinogenic activities. It is well recognized that the presence of phosphoryl or thiophosphoryl group in a molecule can control molecular replication, cell biochemistry and metabolic processes in all living species¹³ and acts as phosphorylating agent of enzymes possessing good biological activity.

Piperidines are essential scaffolds in numerous natural products,¹⁴ synthetic pharmaceuticals¹⁵ and wide variety of biological compounds.¹⁶ It has been found that compounds containing piperidine moiety have entered preclinical and clinical trials¹⁷ over the last few years. Further, the piperidine derivatives have exhibited diverse biological activities like antibacterial, antimalarial, anti-inflammatory and enzyme inhibitory. Numerous methodologies have also been developed using piperidine moiety involving various cyclization techniques.¹⁸ For example, substituted 2-amino methyl

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piperidine is found to be the main class of selective protein kinase C, and has emerged as a key mediator in cellular regulation, signal transduction and neoplastic promotion.¹⁹

Nucleosides and their analogues are one of the important compounds that are largely studied and used as antiviral and antitumour agents.²⁰ It was recognized that some of the antiviral drugs are able to mimic natural nucleosides and as such serve as building units or inhibitors that inhibit nucleic acid synthesis or block nucleoside/nucleotide dependent biological process.²¹⁻²⁵ Further, some of the nucleoside derivatives exhibit moderate to good activity against specific bacterial strains. But for the last two decades, the studies on antibacterial properties have often been ignored in favour of those relating to antiviral and anti tumour activities. Yet, some recent experiences have proved the great value of this field of research. For example, complex nucleosides have showed signs of antibacterial activity by specific inhibition of cell wall peptidoglycon biosynthesis,²⁶ DNA ligases,²⁷ adenosine analogues such as cordycepin²⁸ and some purine derivatives²⁹ have shown biological activity including antimicrobial properties.

In general, nucleosides phosphorylated at C-5 position have been used as biological probes in the study of various enzyme-catalyzed reactions.³⁰⁻³² These nucleosides become bioactive depending on the ability to convert to their nucleotides, thereby establishing their biological function in the inhibition of viral replication and cell proliferation through inhibition of enzymes involved in the de novo biosynthesis of nucleotides.³³⁻³⁵ Thus, nucleoside analog chemotherapy has become crucial in the treatment of hematological and solid malignances.³⁶ Further, the use of nucleosides continued to dominate over other drugs for the treatment of various diseases such as HIV, HBV, cancer,^{37, 38} viral^{39, 40} and act as target prodrugs for the quantifiable remedy for the treatment of AIDS.^{36, 41} Recent studies, have proved that introduction of various nucleosides to the P-N functional moieties increases cellular uptake and enhances the chemotherapeutic properties.⁴²

By finding the importance of P-heterocyclics and nucleosides in biology from literature survey and our continuous research on development of phosphorus biomolecules,⁴³ herein, we synthesized nucleoside substituted perhydro-1 λ 5-[1,3,2]diazaphospholo[1,5-*a*]pyridine-3-thione derivatives **4(a-j)**.

2. Experimental

2.1. Chemical Material and Apparatus

All chemicals were purchased from Merck, Aldrich and S. D. Fine-chem. (India) for use without further purification. Solvents were distilled over with appropriate drying agents and degassed before use. Melting points were determined in open capillaries using Guna melting point apparatus and are uncorrected. IR spectra were recorded on JASCO FT-IR 5300 by pressed pellet method using KBr. ¹H NMR, ¹³C NMR and ³¹P NMR spectra were recorded on Bruker AV-400 spectrometer operating at 400 MHz for ¹H NMR, 100.6 MHz for ¹³C NMR and 161.9 MHz for ³¹P NMR in DMSO-*d*₆. Tetramethylsilane (TMS) and 85% H₃PO₄ were used as internal and external standards to record ¹H, ¹³C NMR and ³¹P MR spectra, respectively. Mass spectra were recorded on API3000 mass spectrometer in positive mode. The progress of the reactions was monitored by TLC on Merck silica plates. Hi-media silica gel (60-120 mesh) was used for column chromatography. Results are presented as chemical shift δ in ppm, multiplicity, *J* values in Hertz (Hz), number of protons, proton's position. Multiplicities are shown as the abbreviations: s (singlet), d (doublet), t (triplet), m (multiplet).

2.2. Synthesis of Compounds

2.2.1. General Procedure for the Synthesis of Nucleoside Substituted Perhydro-1 λ 5-[1,3,2]Diazaphospholo[1,5-*a*]pyridine-3-thione Derivatives **4(a-j)**

A solution of thiophosphoryl chloride **2** (0.10 mL, 1.0 mmol) in 5 mL of dry THF was added drop wise over a period of 20 min to a stirred solution of 2(±)-aminomethylpiperidine (**1**) (0.12 mL, 1.0 mmol) and triethylamine (0.27 mL, 0.2 mmol) at -5–0 °C for 1 h. After addition was completed, reaction mixture was stirred for 1 h by slowly raising the temperature to ambient temperature. The completion of reaction monitored by TLC (ethyl acetate and hexane, 3:7), reaction mixture was

filtered off to remove salt, $\text{Et}_3\text{N}\cdot\text{HCl}$. The filtrate containing monochloride intermediate, 1-chloroperhydro- $1\lambda^5$ -[1,3,2]diazaphospholo[1,5-a]pyridine-1-thione **3** was used in next step without any purification to obtain the title compounds.

To the intermediate **3**, Zidovudine **4b** (0.267g, 1.0 mmol, in 6 mL of THF: Py (2:1) and triethylamine (0.12 mL, 2.0 mmol) were added and heated to reflux (55-60 °C) the reaction mixture and then stirred for 6 h. The progress of reaction was monitored by TLC using methanol and dichloromethane (1:9) as an eluent. After completion of the reaction, $\text{Et}_3\text{N}\cdot\text{HCl}$ was filtered off and the residue was washed twice with 4 mL of THF. The combined organic layer was concentrated under vacuum and the crude product was subjected to column chromatography (60-120 silica mesh) using initially with the mixture of cyclohexane and ethyl acetate (2:8), and then mixture of methanol and ethylacetate (1:9) as an eluent systems to obtain the pure product, 1-(4-azido-5-[(1-thioxoperhydro- $1\lambda^5$ -[1,3,2]diazaphospholo[1,5-a]pyridine-1-yl)oxy]methyl}tetrahydro-2-furanyl)-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione **4b** in 78% yield. The same procedure was adopted for synthesizing the remaining title compounds.

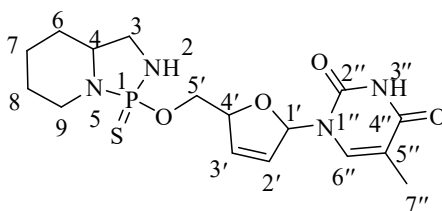


Figure 1. Numbering the title compound (**4a**)

2.2.2. *Methyl-1-[5-[(1-thioxoperhydro- $1\lambda^5$ -[1,3,2]diazaphospholo[1,5-a]pyridin-1-yl)oxy]methyl}-2,5-dihydro-2-furanyl]-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**4a**):* Yield: 74%; Reddish brown solid, m.p: 170-172 °C; IR (KBr): ν 775, 1697, 3402; ^1H NMR (DMSO- d_6): δ 1.10-1.35 (m, 6H, H-9, H-8, H-7), 2.08 (t, 2H, H-6), 2.47-2.52 (m, 2H, H-3), 2.67 (s, 3H, H-7''), 3.44 (s, 1H, H-2), 3.67-3.70 (m, 1H, H-4), 3.92 (d, 2H, $J = 7.2$, H-5'), 4.02-4.06 (m, 1H, H-4'), 5.80-5.94 (m, 2H, H-2', H-3'), 6.56 (d, 1H, $J = 8.1$, H-1'), 7.27 (s, 1H, H-6''), 10.68 (s, 1H, H-3''); ^{13}C NMR (DMSO- d_6): δ 13.9, 21.8, 28.5, 31.7, 46.7, 51.3, 58.3, 67.4, 85.0, 90.9, 114.1, 128.5, 131.6, 139.3, 153.4, 168.4; ^{31}P NMR (DMSO- d_6): δ :0.3; MS (m/z): 399 (M+H) $^+$.

2.2.3. *1-(4-Azido-5-[(1-thioxoperhydro- $1\lambda^5$ -[1,3,2]diazaphospholo[1,5-a]pyridin-1-yl)oxy]methyl}tetrahydro-2-furanyl)-5-methyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**4b**):* Yield: 78%; Reddish brown solid, m.p: 202-204 °C; IR (KBr): ν 765, 1685, 3423; ^1H NMR (DMSO- d_6): δ 1.10-1.35 (m, 4H, H-9, H-8), 1.83-1.94 (m, 3H, H-7, H-3'), 2.32-2.56 (m, 2H, H-2'), 2.64 (s, 3H, H-7''), 2.72-2.91 (m, 4H, H-3, H-6), 3.65 (s, 1H, H-2), 3.68-3.71 (m, 1H, H-4), 3.91 (d, 2H, $J = 7.6$, H-5'), 4.43 (t, 1H, $J = 7.6$, H-4'), 6.06 (t, 1H, $J = 7.2$, H-1'), 7.24 (s, 1H, H-6''), 9.74 (s, 1H, H-3''); ^{13}C NMR (DMSO- d_6): δ 13.6, 21.2, 28.4, 31.5, 43.8, 46.7, 52.1, 57.7, 58.4, 64.5, 82.9, 87.9, 112.8, 134.7, 152.9, 164.6; ^{31}P NMR (DMSO- d_6): δ :0.4; MS (m/z): 442.3 (M+H) $^+$.

2.2.4. *4-Amino-1-(2-[(1-thioxoperhydro- $1\lambda^5$ -[1,3,2]diazaphospholo[1,5-a]pyridin-1-yl)oxy]methyl-1,3-oxathiolan-5-yl)-1,2-dihydro-2-pyrimidinone (**4c**):* Yield: 72%; Brown solid, m.p: 187-189 °C; IR (KBr): ν 777, 1682, 3350; ^1H NMR (DMSO- d_6): δ 1.14-1.28 (m, 4H, H-9, H-8), 1.58-1.73 (m, 2H, H-7), 2.84-2.93 (m, 2H, H-6), 2.98-3.08 (m, 4H, H-3, H-2'), 3.24 (s, 1H, H-2), 3.52-3.62 (m, 1H, H-4), 3.94 (t, 1H, $J = 7.2$, H-4'), 4.21 (t, 2H, $J = 7.6$, H-5'), 5.28 (d, 1H, $J = 6.8$, H-1'), 6.74 (d, 1H, $J = 7.6$, H-5''), 6.82 (s, 2H, H-7''), 8.63 (d, 1H, $J = 7.6$, H-6''); ^{13}C NMR (DMSO- d_6): δ 24.1, 27.9, 30.3, 32.1, 47.2, 50.0, 56.9, 62.7, 86.1, 93.7, 99.9, 141.2, 154.6, 165.6; ^{31}P NMR (DMSO- d_6): δ 1.3. MS (m/z): 404 (M+H) $^+$.

2.2.5. *1-[6-(6-amino-9H-9-purinyl)-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl]methoxyperhydro- $1\lambda^5$ -[1,3,2]diazaphospholo[1,5-a]pyridine-1-thione (**4d**):* Yield: 68%, Dark red solid, m.p: 192-194 °C; IR (KBr): ν 720, 3300; ^1H NMR (DMSO- d_6): δ 1.62-1.68 (m, 4H, H-9, H-8), 1.70-1.78

(m, 2H, H-7), 1.99 (s, 6H, H-7', H-8'), 2.08-2.19 (m, 2H, H-6), 2.49-3.51 (m, 2H, H-3), 3.38 (s, 1H, H-2), 3.60-3.67 (m, 1H, H-4), 3.74 (t, 2H, $J = 6.4$, H-5'), 4.32-4.38 (m, 3H, H-4', H-3', H-2'), 6.23 (d, 1H, $J = 6.0$, H-1'), 6.32 (s, 2H, H-10''), 8.18 (s, 1H, H-3''), 8.33 (s, 1H, H-2''); ^{13}C NMR (DMSO- d_6): δ 21.9, 23.7, 27.4, 29.7, 44.3, 49.3, 54.5, 68.9, 84.6, 87.4, 89.9, 97.9, 109.9, 119.3, 149.8, 154.7, 158.2, 161.0; ^{31}P NMR (DMSO- d_6): δ -1.7; MS (m/z): 482 (M+H) $^+$.

2.2.6. 9-(5- $\{[(1\text{-Thioxoperhydro-}1\lambda^5\text{-}[1,3,2]\text{diazaphospholo}[1,5\text{-a]pyridin-1-yl)oxy}\}methyl\}$ tetrahydro-2-furanyl)-6,9-dihydro-3H-6-purinone (**4e**): Yield: 65%; Brown solid, m.p: 195-197 °C; IR (KBr): ν 750, 1714, 3434; ^1H NMR (DMSO- d_6): δ 1.34-1.44 (m, 4H, H-9, H-8), 1.54-1.60 (m, 2H, H-7), 1.96-2.04 (m, 2H, H-3'), 2.21-2.28 (m, 2H, H-2'), 2.43-2.48 (m, 4H, H-3, H-6), 2.96 (s, 1H, H-2), 3.08 (s, 1H, H-3''), 3.26-3.36 (m, 1H, H-4), 3.74 (d, 2H, $J = 7.2$, H-5'), 3.96-4.02 (m, 1H, H-4'), 4.98 (t, 1H, $J = 6.4$, H-1'), 7.83 (s, 1H, H-8''), 7.89 (s, 1H, H-2''); ^{13}C NMR (DMSO- d_6): δ 24.6, 28.2, 28.6, 31.4, 32.4, 43.2, 49.1, 54.5, 56.4, 60.9, 74.2, 93.6, 117.2, 157.4, 160.7, 186.1; ^{31}P NMR (DMSO- d_6): δ 0.3; MS (m/z): 411 (M+H) $^+$.

2.2.7. 1-[2-(6-amino-9H-9-puriny)-1-methylethoxy]perhydro-1 λ^5 -[1,3,2]diazaphospholo[1,5-a]pyridine-1-thione (**4f**): Yield: 67%; Reddish brown solid, m.p: 199-201 °C; IR (KBr): ν 777, 3350; ^1H NMR (DMSO- d_6): δ 1.27 (d, 3H, $J = 8.4$, H-3'), 1.35-1.41 (m, 4H, H-9, H-8), 1.54-1.62 (m, 2H, H-7), 2.51-2.58 (m, 4H, H-3, H-6), 2.91 (s, 1H, H-2), 3.24-3.29 (m, 1H, H-4), 3.74 (t, 1H, $J = 6.4$, H-2'), 4.16 (d, 2H, $J = 7.2$, H-1'), 6.92 (s, 2H, H-10''), 8.13 (s, 1H, H-8''), 8.28 (s, 1H, H-2''); ^{13}C NMR (DMSO- d_6): δ 21.6, 23.2, 27.5, 32.6, 43.3, 46.8, 57.1, 58.4, 62.6, 121.8, 146.1, 150.7, 154.3, 158.3; ^{31}P NMR (DMSO- d_6): δ -1.6; MS (m/z): 368 (M+H) $^+$.

2.2.8. 1-(3,4-Dihydroxy-5- $\{[(1\text{-thioxoperhydro-}1\lambda^5\text{-}[1,3,2]\text{diazaphospholo}[1,5\text{-a]pyridin-1-yl)oxy}\}methyl\}$ tetrahydro-2-furanyl)-1H-1,2,4-triazole-3-carboxamide (**4g**): Yield: 72%; Reddish solid, m.p: 191-193 °C; IR (KBr): ν 796, 1685, 3223, 3402; ^1H NMR (DMSO- d_6): δ 1.21-1.25 (m, 3H, H-8, H-7), 1.44-1.55 (m, 3H, H-9, H-7), 2.49-2.50 (m, 4H, H-3, H-6), 3.20 (s, 1H, H-2), 3.33-3.36 (m, 1H, H-4), 3.71 (d, 2H, $J = 12.5$, H-5'), 4.88-5.00 (m, 3H, H-2', H-3', H-4'), 5.77 (s, 2H, H-6', H-7'), 6.65 (d, 1H, $J = 4.8$, H-1'), 7.92 (s, 2H, H-7''), 8.11 (s, 1H, H-5''); ^{13}C NMR (DMSO- d_6): δ 21.9, 28.9, 31.4, 48.6, 51.0, 55.9, 59.4, 72.4, 80.1, 88.9, 94.5, 145.9, 152.6, 166.3; ^{31}P NMR (DMSO- d_6): δ 3.4; MS (m/z): 419 (M+H) $^+$.

2.2.9. 4-Amino-5-fluoro-1-(2- $\{[(1\text{-thioxoperhydro-}1\lambda^5\text{-}[1,3,2]\text{diazaphospholo}[1,5\text{-a]pyridin-1-yl)oxy}\}methyl\}$ 1,3-oxathiolan-5-yl)-1,2-dihydro-2-pyrimidinone (**4h**): Yield: 75%; Dark red solid, m.p: 181-183 °C; IR (KBr): ν 780, 1680, 3449; ^1H NMR (DMSO- d_6): δ 1.13-1.22 (m, 4H, H-9, H-8), 1.32-1.48 (m, 2H, H-7), 2.68-2.79 (m, 2H, H-6), 2.88-2.98 (m, 4H, H-3, H-2'), 3.15 (s, 1H, H-2), 3.52-3.59 (m, 1H, H-4), 3.86 (t, 1H, $J = 7.6$, H-4'), 4.32 (t, 2H, $J = 7.6$, H-5'), 5.41 (d, 1H, $J = 6.4$, H-1'), 6.26 (s, 2H, H-7''), 8.01 (d, 1H, $J = 8.8$, H-6''); ^{13}C NMR (DMSO- d_6): δ 24.2, 28.6, 29.7, 32.4, 45.5, 49.1, 55.3, 64.1, 86.1, 93.7, 108.6, 132.1, 154.4, 158.9; ^{31}P NMR (DMSO- d_6): δ 3.4; MS (m/z): 422 (M+H) $^+$.

2.2.10. 4-Amino-1-(3,4-dihydroxy-5- $\{[(1\text{-thioxoperhydro-}1\lambda^5\text{-}[1,3,2]\text{diazaphospholo}[1,5\text{-a]pyridin-1-yl)oxy}\}methyl\}$ tetrahydro-2-furanyl)-1,2-dihydro-2-pyrimidinone (**4i**): Yield: 69%; Red solid, m.p: 207-209 °C; IR (KBr): ν 760, 1697, 3343, 3412; ^1H NMR (DMSO- d_6): δ 1.43-1.49 (m, 4H, H-9, H-8), 1.57-1.68 (m, 2H, H-7), 2.54-2.61 (m, 4H, H-3, H-6), 3.09 (s, 1H, H-2), 3.52-3.58 (m, 1H, H-4), 3.64 (d, 2H, $J = 7.6$, H-5'), 4.49-4.58 (m, 2H, H-2', H-3'), 4.61-4.65 (m, 1H, H-4'), 4.93 (s, 2H, H-6', H-7'), 5.89 (d, 1H, $J = 6.8$, H-1'), 6.01 (d, 1H, $J = 7.4$, H-5''), 7.43 (s, 2H, H-7''), 8.67 (d, 1H, $J = 7.4$, H-6''); ^{13}C NMR (DMSO- d_6): δ 23.6, 28.2, 34.7, 49.4, 49.6, 57.6, 61.8, 74.1, 82.6, 86.1, 93.6, 102.4, 142.8, 158.4, 160.4; ^{31}P NMR (DMSO- d_6): δ 2.3; MS (m/z): 418 (M+H) $^+$.

2.2.11. 9-(3,4-Dihydroxy-5- $\{[(1\text{-thioxoperhydro-}1\lambda^5\text{-}[1,3,2]\text{diazaphospholo}[1,5\text{-a]pyridin-1-yl)oxy}\}methyl\}$ tetrahydro-2-furanyl)-6,9-dihydro-1H-6-purinone (**4j**): Yield: 65%; Red solid, m.p: 190-192 °C; IR (KBr): ν 740, 1750, 3253, 3408; ^1H NMR (DMSO- d_6): δ 1.36-1.43 (m, 4H, H-9, H-8),

1.49-1.54 (m, 2H, H-7), 2.48-2.57 (m, 4H, H-3, H-6), 3.11 (s, 1H, H-2), 3.37-3.49 (m, 1H, H-4), 3.86 (d, 2H, $J = 8.0$, H-5'), 4.53-4.65 (m, 3H, H-2', H-3', H-4'), 4.87 (s, 2H, H-6', H-7'), 6.32 (d, 1H, $J = 5.6$, H-1'), 7.89 (s, 1H, H-2''), 7.92 (s, 1H, H-8''), 10.06 (s, 1H, H-1''); ^{13}C NMR (DMSO- d_6): δ 23.1, 28.5, 33.6, 45.8, 48.4, 58.3, 64.8, 74.9, 78.6, 83.4, 91.2, 128.7, 138.2, 148.8, 151.4, 158.6; ^{31}P NMR (DMSO- d_6): δ -0.3. MS (m/z): 443 (M+H) $^+$.

2.3. Biological Assay

2.3.1. Antiviral Activity

The Lasota strain of NDV virus was collected from the Department of Virology, Sri Venkateswara University, Tirupati. The titre of the NDV virus was measured as 1/128. The embryonated chicken eggs used for the experiment were collected from the poultry division, Sri Venkateswara Veterinary University, Tirupati and incubated in an incubator at 37 °C. Antiviral assay of all the newly synthesized compounds **4(a-j)** was tested against Newcastle Disease Virus (NDV) using allantoic sac of developing chicken embryos with some minor modifications (Rajbhandari *et al.*, 2001)⁴⁵⁻⁴⁶. Nine-eleven days old chicken embryos were labeled according to the compounds used. The eggs were swabbed with 70% alcohol in a cotton wool and transferred into a cleaned tray. The swabbed eggs were placed in micro safety cabinet, punched and inoculated with the compound/virus mixture via the allantoic sac route. All the titled compounds dissolved in DMSO were treated with a suspension of 0.1 mL of the NDV virus at 1 mg/mL of the compound. DMSO was used as negative control, it doesnot affect the test organisms. The treated virus was incubated at 4 °C for 1 h. Then, the treated viruses were inoculated through allantoic sac route of 9-11 days old chick embryos. PBS solution was used as a control. The eggs were sealed with molten wax and incubated at 37 °C. Allantoic fluid from the treated eggs was collected for the haemagglutination test to detect NDV in the eggs.

2.3.2. Haemagglutination Test (HA)

50 μL PBS solution (0.01M, pH 7.2) and 50 μL of the compound, 50 μL of the freshly prepared 1% washed chicken red blood cells (RBCs) were added to each well of U-shaped micro titre plates with the help of micro pipette calibrated with 25-200 μL and were incubated at room temperature for 30 min. The results were recorded after 30 min of incubation and are presented in Table 1.

2.3.2. Antibacterial Activity

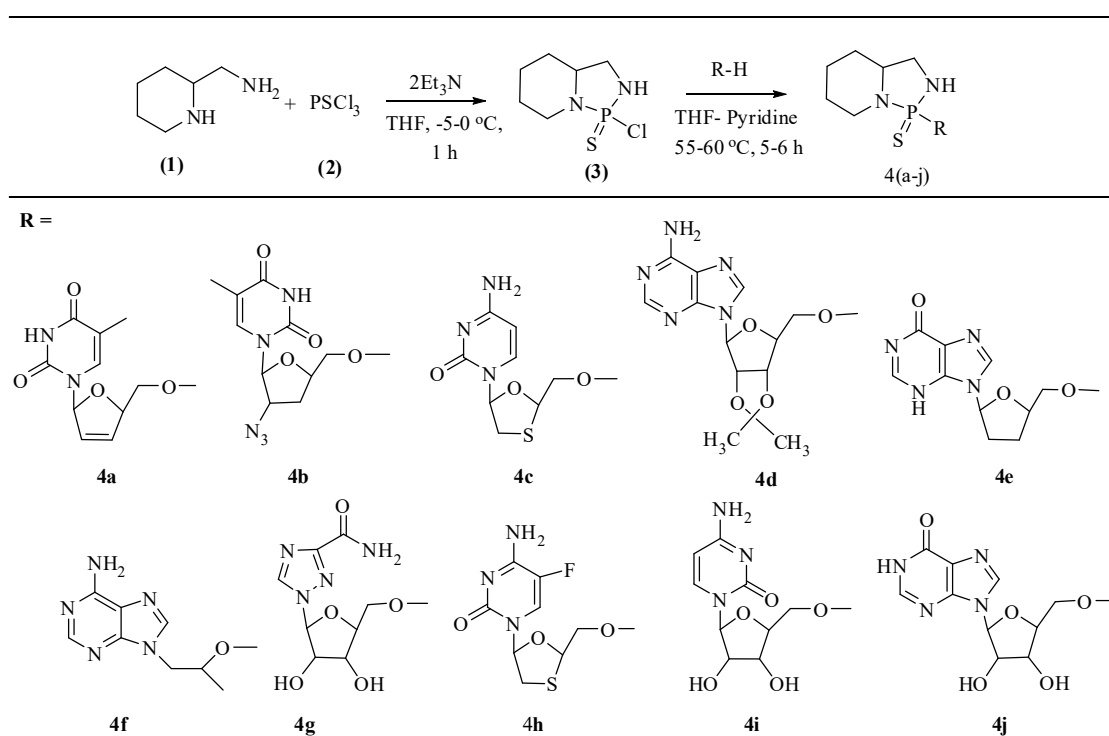
In vitro antibacterial activity of the newly synthesized compounds was carried out using agar disc diffusion method^{47,48} on nutrient agar medium against *Bacillus subtilis* (ATCC-6051), *Bacillus pumilis* (MTCC-2296), *Pseudomonas marginalis* (MTCC-2758), and *Pseudomonas aeruginosa* (ATCC-27853). The nutrient agar medium in each petri plate was homogenously spread with a bacterial strains and incubated at 25 \pm 2 °C for 24 h. All the test compounds were dissolved in DMSO. DMSO was used as negative control, it doesnot affect the test organisms. Sterile disc of 6 mm whatmann filter No.1 was introduced onto the media. 200 $\mu\text{g}/\text{mL}$ of the test compound was introduced onto the disc and dried completely to saturate the disc with the test compound and incubated at 25 \pm 2 °C for 24 h. After incubation, zone of inhibition around the disc was calculated edge to edge zone of the disc and was measured in millimeters. All the tests were carried out three times and average was taken as the final result. Ciprofloxacin was used as a standard drug and zone of inhibition of the test compounds were compared with the standard controls and results are presented in Table 2.

2.3.3. Antifungal Activity

Newly synthesized compounds were evaluated against *Aspergillus niger* (MTCC-1881), *Fusarium oxysporum* (MTCC-1755) and *Candida albicans* (ATCC-2091) using poison plate method⁴⁹ and Ketoconazole was used as a standard drug. All the newly synthesized compounds were dissolved in DMSO separately and were transferred into the sterile petri dishes at concentration 200 µg/mL using micropipette. Potato dextrose agar (PDA) medium and the sterile PDA plate were prepared. 0.1 mL of each fungal strain was spread on each plate and incubated at 27±2 °C for 24 h. After proper preparation of incubation well using cork borer, each agar well is filled with 0.1 mL of compound solution at concentration 200 µg/mL. The plates were kept in a refrigerator for 20 min for diffusion and incubated at 27± 2 °C for 5 days. DMSO was used as negative control, it doesnot affect the test organisms. Inhibition zones of the titled compounds were measured and are presented in Table 3.

3. Results and Discussion

The synthesis of nucleosides substituted perhydro-1λ⁵-[1,3,2]diazaphospholo[1,5-a]pyridine-3-thione derivatives **4(a-j)** have been carried out as per the reactions formulated in Scheme 1



Scheme 1. Synthesis of nucleoside substituted perhydro-1λ⁵-[1,3,2]diazaphospholo[1,5-a]pyridine-3-thione derivatives **4(a-j)**

Initially, the cyclocondensation of 2(±)-aminomethylpiperidine (**1**) with thiophosphyryl chloride (**2**) was carried out in the presence of triethylamine (Et₃N) at -5–0 °C for 1 h to form a key intermediate, 1-chloroperhydro-1λ⁵-[1,3,2]diazaphospholo[1,5-a]pyridine-1-thione (**3**) which was ascertained by thin layer chromatography (TLC). The salt Et₃N·HCl, after completion of the reaction, was removed from the reaction mixture by filtration and the filtrate containing an intermediate (**3**) was used for the next step of the reaction without any further purification. Secondly, various nucleosides in the presence of Et₃N /Pyridine were added to the filtrate containing an intermediate (**3**) and stirred at 55–60 °C to obtain the title compounds, nucleosides substituted perhydro-1λ⁵-[1,3,2]diazaphospholo[1,5-a]pyridine-3-thione derivatives **4(a-j)**. The final crude compounds were obtained by the filtration of Et₃N·HCl followed by concentrated under vacuum. The compounds were

purified by subjecting to the column chromatography using initially the mixture of cyclohexane and ethyl acetate (2:8), and then mixture of methanol and ethylacetate (1:9) as an eluent systems.

Structures of all the newly synthesized compounds **4(a-j)** were confirmed by spectroscopic data (^1H , ^{13}C , ^{31}P NMR and IR) and mass spectrophotometry. In IR spectra, the appearance of absorption bands in the regions 3253-3449, 1680-1750 and 720-796 cm^{-1} confirmed the functionalities -NH, C=O and P=S, respectively. ^1H NMR chemical shifts values are observed in the regions 1.42-1.60, 2.47-3.08, 2.91-3.65, 3.26-3.71, and 3.64-4.21 ppm confirmed the protons of piperidine ring. Further, ^{13}C NMR chemical shift values for aliphatic carbons of piperidine were resonated in the region δ 21.2-58.3 and the carbonyl carbons in the region δ 152.9-164.6 ppm. The mass spectra of the compounds showed molecular ion at appropriate m/z values, which correspond well to the respective molecular formula and HRMS were recorded for the all compounds.

All the newly synthesized compounds **4(a-j)** were evaluated for their antiviral activity against Newcastle disease (NDV) virus using allantoic sac of embryonated eggs and haemagglutination (HA) tests and the results are summarized in **Table 1**. The bio-efficiency of the final compounds **4(a-j)** was screened against NDV virus in embryonated chicken eggs in time dependent fashion (until 4 days). Haemagglutination test was carried out to associate embryo mortality with viral activity. The standard doxorubicin was used as positive control to compare the antiviral activity of the title products. The observations in the course study of antiviral activity revealed that all the newly synthesized compounds **4(a-j)** showed no embryo death (mortality) when inoculated with NDV at 24 h post inoculation. Compounds **4a**, **4b**, **4e**, **4g**, **4h** show no embryo death at 48 h post inoculation. All five NDV infected embryonated eggs in compound **4b** had survived even after 72 h (zero mortality) of post inoculation which is equivalent activity to that exhibited by the standard drug.

Table 1. Antiviral activity of nucleoside substituted perhydro-1 λ^5 -[1,3,2]diazaphospholo[1, 5-*a*]pyridine-3-thione derivatives **4(a-j)**

| Compound | No. of Eggs | Mortality | | | | HA Test | |
|--------------------|-------------|-----------|------|------|------|---------|-----|
| | | 24 h | 48 h | 72 h | 96 h | +ve | -ve |
| 4a | 5 | 0/5 | 0/5 | 1/5 | 2/4 | 3 | 2 |
| 4b | 5 | 0/5 | 0/5 | 0/5 | 2/5 | 2 | 3 |
| 4c | 5 | 0/5 | 1/5 | 0/4 | 2/4 | 3 | 2 |
| 4d | 5 | 0/5 | 1/5 | 2/4 | 1/2 | 4 | 1 |
| 4e | 5 | 0/5 | 0/5 | 1/5 | 2/4 | 3 | 2 |
| 4f | 5 | 0/5 | 1/5 | 2/4 | 1/2 | 4 | 1 |
| 4g | 5 | 0/5 | 0/5 | 1/5 | 2/4 | 3 | 2 |
| 4h | 5 | 0/5 | 0/5 | 1/5 | 1/4 | 2 | 3 |
| 4i | 5 | 0/5 | 1/5 | 2/4 | 1/2 | 4 | 1 |
| 4j | 5 | 0/5 | 1/5 | 0/4 | 2/4 | 3 | 2 |
| *PBS | 5 | 0/5 | 0/5 | 0/5 | 0/5 | 0 | 0 |
| Doxorubicin | 5 | 0/5 | 0/5 | 0/5 | 1/5 | 0 | 0 |

*PBS: Phosphate Buffered Saline

Antimicrobial potency of the newly synthesized title products **4(a-j)** was tested against four bacterial strains such as *Bacillus subtilis* (ATCC-6051), *Bacillus pumilis* (MTCC-2296), *Pseudomonas marginalis* (MTCC-2758) and *Pseudomonas aeruginosa* (ATCC-27853), and fungal strains such as *Aspergillus niger* (MTCC-1881), *Fusarium oxysporum* (MTCC-1755) and *Candida albicans* (ATCC-2091). Agar disc diffusion method and poison plate technique method has been used to test antibacterial and antifungal activities respectively. Ciprofloxacin in antibacterial activity and Ketoconazole in antifungal activity were used as the standards (positive control) to compare the title products activity.

The results of antibacterial activity of all the newly synthesized compounds **4(a-j)** are evaluated in Table 2. Most of the compounds have shown significant level of activity in comparison with the standard used. Compounds **4b** linked with zidovudine and **4c** bonded with lamivudine showed highest activity against all the tested pathogens. Compound **4e** against *P. marginalis* and

compound **4h** against *B. subtilis* showed highest activity when compared to the remaining title compounds.

Table 2. Antibacterial activity of nucleoside substituted perhydro-1λ⁵-[1,3,2]diazaphospholo[1,5-*a*]pyridine-3-thione derivatives **4(a-j)**

| Compound | Zone of inhibition (in mm) at 200µg/mL | | | |
|----------------------|--|-------------------|----------------------|----------------------|
| | <i>B. subtilis</i> | <i>B. pumilis</i> | <i>P. marginalis</i> | <i>P. aeruginosa</i> |
| 4a | 18.5±0.94 | 16±0.72 | 19.5±0.85 | 17±0.65 |
| 4b | 21±1.05 | 25±0.89 | 20.5±0.91 | 22.5±1.15 |
| 4c | 24.5±0.86 | 21.5±0.91 | 21±1.02 | 20.5±1.15 |
| 4d | 17±0.75 | 19±0.67 | 18.5±0.79 | 16.5±0.58 |
| 4e | 16.5±0.69 | 18.5±0.71 | 22.5±1.05 | 19±0.73 |
| 4f | 18±0.81 | 17±0.68 | 16.5±0.65 | 15±0.85 |
| 4g | 15.5±0.62 | 16.5±0.57 | 14±0.92 | 17.5±0.57 |
| 4h | 20.5±0.97 | 19±0.73 | 17±0.87 | 18±0.65 |
| 4i | 15±0.58 | 16.5±0.65 | 14.5±0.74 | 17±0.52 |
| 4j | 17.5±0.64 | 18±0.58 | 16±0.70 | 19.5±0.83 |
| Ciprofloxacin | 26±1.32 | 27±1.20 | 24±1.52 | 25±1.41 |

*Values are in millimeter (mm)

Values are expressed as mean (n=3) and analyzed by ANOVA

The results of antifungal activity of all the newly synthesized compounds **4(a-j)** are evaluated in Table 3. Most of the compounds exhibited significant level of activity against the tested pathogens in comparison to the standard. Compounds **4b** linked with zidovudine, **5e** bonded with didanosine and **4i** connected with cytidine exhibited highest activity against all the tested pathogens. Compound **4c** exhibited highest activity against *F. oxysporum* when compared with remaining title compounds.

Table 3. Antifungal activity of nucleoside substituted perhydro-1λ⁵-[1,3,2] diazaphospholo[1,5-*a*]pyridine-3-thione derivatives **4(a-j)**

| Compound | Zone of inhibition (in mm) at 200µg/mL | | |
|---------------------|--|---------------------|--------------------|
| | <i>A. niger</i> | <i>F. oxysporum</i> | <i>C. albicans</i> |
| 4a | 17.5±0.58 | 16±0.75 | 15.5±0.94 |
| 4b | 22±1.35 | 23.5±0.97 | 20.5±0.59 |
| 4c | 18±0.73 | 21.5±1.07 | 17±0.82 |
| 4d | 17.5±0.65 | 16.5±0.84 | 18.5±0.67 |
| 4e | 25.5±1.5 | 22±1.17 | 21.5±0.96 |
| 4f | 16.5±0.82 | 17.5±0.95 | 19±1.05 |
| 4g | 14±0.95 | 16.5±0.88 | 15±0.95 |
| 4h | 17±0.73 | 19±1.02 | 18.5±0.78 |
| 4i | 24±1.05 | 20.5±0.93 | 21±1.07 |
| 4j | 18.5±0.91 | 17±0.77 | 16.5±0.59 |
| Ketoconazole | 27±1.42 | 25±1.52 | 24±1.35 |

*Values are in millimeter (mm);

Values are expressed in mean (n=3) and analyzed by ANOVA

In whole biological observations, the title products, nucleosides substituted perhydro-1λ⁵-[1,3,2]diazaphospholo[1,5-*a*]pyridine-3-thione derivatives **4(a-j)** are acted as potent antiviral agents as compared with antimicrobial agents. However, zidovudine linked derivative, **4b** exhibited highest (antiviral and antimicrobial) activities.

4. Conclusion

We have synthesized a new series of nucleoside substituted perhydro-1 λ^5 -[1,3,2]diazaphospholo[1,5-*a*]pyridine-3-thione derivatives and evaluated for their antiviral and antimicrobial activity against the growth of selected microorganisms. In the biological assay, compounds **4b**, **4h** showed highest antiviral activity against NDV and **4b**, **4c**, exhibited highest antibacterial activity and **4b**, **4e**, **4i** showed highest antifungal activity. Overall, nucleoside, compound **4b** bearing zidovudine has shown highest (antiviral and antimicrobial) activities. This work may be beneficial to develop chemotherapeutic value of the nucleoside derivatives and good impact to medicinal and phosphorus chemistry.

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References

- [1] Kafarski, P.; Lejczak, B. Aminophosphonic acids of potential medical importance. *Curr. Med. Chem. Anti-Canc. Agents.* **2001**, *1*, 301-312.
- [2] Rein, T.; Pedersen, T. M. Asymmetric Wittig type reactions. *Synthesis* **2002**, *5*, 579-594.
- [3] McReynolds, M. D.; Dougherty, J. M.; Hanson, P. R. Synthesis of phosphorus and sulfur heterocycles via ring-closing olefin metathesis. *Chem. Rev.* **2004**, *104*, 2239-2258.
- [4] Abdel-Rahman, R. M. Synthesis of New Phosphaheterobicyclic systems containing 1,2,4-Triazine moiety Part IX; Straightforward Synthesis of New Fluorine Bearing 5-Phospha-1,2,4-triazin/1,2,4-triazepine-3-thions-part X. *Trends Heterocycl. Chem.* **2002**, *8*, 187-195.
- [5] He, L. N.; Zhuo, R. X.; Chen, R. Y.; Li, K.; Zhang, Y. Synthesis of biologically active phosphorus heterocycles via cyclization reactions of Lawesson's reagent. *J. Heteroat. Chem.* **1999**, *10*, 105-111.
- [6] Eugenia, F. C.; Laichici, M.; Gheroghe, F. C.; Vlascici, D. Synthesis, characterization and correlative biological effects in wheat of a benzoxaza- and a diaza-phosphorus(V) heterocycles. *J. Serb. Chem. Soc.* **2006**, *71*, 1031-1038.
- [7] Holla, B. S.; Ashok, M. Convenient synthesis of some thiadiazolotriazinones carrying 4-methylthiobenzyl moieties as possible antimicrobial agents. *Phosphorus, Sulfur, Silicon Relatd. Elem.* **2007**, *182*, 981-991.
- [8] Venugopal, M.; Sankar Reddy, B.; Devendranath Reddy, C.; Berlin, K. D. Synthesis and antimicrobial activity of 2-Substituted-2,3-dihydro-5-propoxy-1H-1,3,2-benzodiazaphosphole 2-Oxides. *J. Heterocycl. Chem.* **2001**, *38*, 275-280.
- [9] Kartizky, A. R.; Rees, C. W.; Scriven, E. F. V. *Comprehensive Heterocyclic Chemistry II*, Pergmona Press, UK, Oxford: **1996**.
- [10] Lemke, T. L.; Boring, D. Novel heterocycles. Synthesis of the 2,4,3-benzodiazaphosphepine ring system. *J. Heterocycl. Chem.* **1980**, *17*, 1455-1456.
- [11] VasuGovardhana Reddy, P.; Haribabu, Y.; Suresh Reddy, C.; Srinivasulu, D. Synthesis of 2-substituted-2,3-dihydro-5-benzoyl-1H-1,3,2-benzodiazaphosphole 2-oxides/sulfides. *Heteroat. Chem.* **2002**, *13*, 340-345.
- [12] Naidu, K. R. M.; Rao, P. V.; Raju, C. N.; Srinivasulu, K. Synthesis and antioxidant activity of substituted-1,3,2-Diazaphosphole 1-Oxides. *Arch. Pharm. Chem. Life Sci.* **2011**, *344*, 765-770.
- [13] Sum, V.; Baird, C. A.; Kee, T. P.; Thomton-Pett, M. [1^N,3^E]-bifunctional phosphorodiamidites and the diastereoselective phosphorylation of aldehydes. Controlling, elucidating and rationalising the

- stereochemical course of the asymmetric Abramov reaction. *J. Chem.Soc., Perkin Trans.* **1994**, *1*, 3183-3200.
- [14] Viegas, C.; Bolzani, J. V. S.; Furlan, M.; Barreiro, E. J.; Young, M. C. M.; Tomazela, D.; Eberlin, M. N. Further bioactive piperidine alkaloids from the flowers and green fruits of *Cassia spectabilis*. *J. Nat. Prod.* **2004**, *67*, 908-910.
- [15] Breman, J.; Egan, A.; Keusch, G. (Editors) The Intolerable Burden of Malaria: A New Look at the Numbers. *Am. J. of Trop. Med. Hygien.* **2001**, suppl. *64*(1),
- [16] Clarke, P. A.; Zaytzev, A. V.; Whitwood, A. C. Pot, atom and step economic (PASE) synthesis of highly functionalized piperidines, a five-component condensation. *Tetrahedron Lett.* **2007**, *48*, 5209-5212.
- [17] Kamei, K.; Maeda, N.; Katsuragi-Ogino, R.; Koyama, M.; Nakajima, M.; Tatsuoka, T.; Ohno, T.; Inoue, T. New piperidinyl- and 1,2,3,6-tetrahydropyridinyl-pyrimidine derivatives as selective 5-HT_{1A} receptor agonists with highly potent anti-ischemic effects. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2990-2993.
- [18] Khan, A. T.; Khan, M. M.; Bannuru, K. K. R. Iodine catalyzed one-pot five-component reactions for direct synthesis of densely functionalized piperidines. *Tetrahedron* **2010**, *66*, 7762-7772.
- [19] Takai, Y.; Kishimoto, A.; Inoue, M.; Nishizuka, Y. Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. *J. Biol. Chem.* **1977**, *252*, 7603-7609.
- [20] Isono, K. Nucleoside antibiotics: structure, biological activity, and biosynthesis. *J. Antibiot.* **1988**, *41*, 1711-1739.
- [21] Declercq, E.; Holy, A. Acyclic nucleoside phosphonates: a key class of antiviral drugs. *Nat. Rev. Drug Discov.* **2005**, *4*, 928-940.
- [22] Galmarini, C. M.; Popowycz, F.; Joseph, B. Cytotoxic nucleoside analogues: different strategies to improve their clinical efficacy. *Curr. Med. Chem.* **2008**, *15*, 1072-1082.
- [23] Berdis, A. J. DNA polymerases as therapeutic targets. *Biochemistry* **2008**, *47*, 8253-8360.
- [24] Ewald, B.; Sampath, D.; Plunkett, W. Nucleoside analogs: molecular mechanisms signaling cell death. *Oncogene* **2008**, *27*, 6522-6537.
- [25] Jordhim, L.P.; Dumontet, C. Review of recent studies on resistance to cytotoxic deoxy nucleoside analogues. *Biochim. Biophys. Acta.* **2007**, *1776*, 138-159.
- [26] Kimura, K.; Bugg, T. D. H. Recent advances in antimicrobial nucleoside antibiotics targeting cell wall biosynthesis. *Nat. Prod. Rep.* **2003**, *20*, 252-273.
- [27] Mills, S. D.; Eakin, A. E.; Buurman, E. T.; Newman, J. V.; Gao, N.; Huynh, H.; Johnson, K. D.; Lahiri, S.; Shapiro, A. B. Novel bacterial NAD⁺-dependent DNA ligase inhibitors with broad-spectrum activity and antibacterial efficacy in vivo. *Antimicrob. Agents Chemother.* **2011**, *55*, 1088-1096.
- [28] Ahn, Y. J.; Park, S. J.; Lee, S. G.; Shin, S. C.; Choi, D. H. Cordycepin: selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp. *J. Agric. Food Chem.* **2000**, *48*, 2744-2748.
- [29] Tunc bilek, M.; Ates-Alago, Z.; Altanlar, N.; Karayel, A.; Ozbey, S. Synthesis and antimicrobial evaluation of some new substituted purine derivatives. *Bioorg. Med. Chem.* **2009**, *17*, 1693-1700.
- [30] De Clercq, E. Toward improved Anti-HIV chemotherapy: Therapeutic strategies for intervention with HIV infections. *J. Med. Chem.* **1995**, *38*, 2491-2517.
- [31] Nair, V.; Jahnke, T. S. Antiviral activities of isomeric dideoxynucleosides of D- and L-related stereochemistry. *Antimicrob. Agents Chemother.* **1995**, *39*, 1017-1029.
- [32] Taktakishvilli, M.; Neamati, N.; Pommier, Y.; Pal, S.; Nair, V. Recognition and inhibition of HIV integrase by novel dinucleotides. *J. Am. Chem. Soc.* **2000**, *122*, 5671-5677.
- [33] Firestein, S. M.; Wu, W. D.; Youn, H.; Davisson, V. Jo. Interrogating the mechanism of a tight binding inhibitor of AIR carboxylase. *Bioorg. Med. Chem.* **2009**, *17*, 794-803.
- [34] Bello, A. M.; Konforte, D.; Poduch, E.; Furlonger, C.; Wei, L. H.; Liu, Y.; Lewis, M.; Pai, E. F.; Paige, C. J.; Kotra, L. P. Structure-activity relationships of orotidine-5'-monophosphate decarboxylase inhibitors as anticancer agents. *J. Med. Chem.* **2009**, *52*, 1648-1658.
- [35] Wang, G. Y.; Sakthivel, K.; Rajappan, V.; Bruce, T. W.; Tucker, K.; Fagan, P.; Brooks, J. L.; Hurd, T.; Leeds, J. M.; Cook, P. D. Synthesis of azole nucleoside 5'-monophosphate mimics (PIMS) and their inhibitory properties of IMP dehydrogenases. *Nucleos. Nucleot. Nucl.* **2004**, *23*, 317-337.
- [36] Clarke, M. L.; Mackey, J. R.; Baldwin, S. A.; Young J. D.; Cass, C. E. The Role of membrane transporters in cellular resistance to anticancer nucleoside drugs. *Cancer Treat. Res.*, **2002**, *112*, 27-47.
- [37] Herdewijn, P. A. M. M. Novel nucleoside strategies for anti-HIV and anti-HSV therapy. *Antiviral Res.* **1992**, *19*, 1-14.
- [38] Browne, M. J.; Mayer, K. H.; Chafee, S. B. D.; Dudley, M. N.; Posner, M. R.; Steinberg, S.M.; Graham, K. K.; Geletko, S. M.; Zinner, S. H.; Denman, S. L.; Dunkle, L. M.; Kaul, S.; McLaren, C.; Skowron, G.; Kouttab, N. M.; Kennedy, T. A.; Weitberg, A. B.; Curt, G. A. 2',3'-Didehydro-3'-deoxythymidine (d4T) in patients with AIDS or AIDS-related Complex: A Phase I Trial. *J. Infect. Dis.* **1993**, *167*, 21-29.

- [39] Murray, H. W.; Squires, K. E.; Weiss, W.; Sledz, S.; Sacks, H. S.; Hassett, J.; Cross, A.; Anderson, R. E.; Dunkle, L. M. Stavudine in patients with aids and aids-related complex: Aids clinical trials group 089. *J. Infect. Dis.* **1995**, *171*, 123-130.
- [40] Baldwin, S. A.; Mackey, J. R.; Cass, C. E.; Young, J. D. Nucleoside transporters: Molecular biology and implications for therapeutic development. *Mol. Med. Today* **1999**, *5*, 216-224.
- [41] Mitsuya, H.; Yarchaon, R.; Broder, S. Molecular targets for AIDS therapy. *Science*, **1990**, *249*, 1533-1544.
- [42] Schelemminger, I.; Wilecke, A.; Maison, W.; Koch, R.; Lutzen, A.; Martens, J. Diastereoselective Lewis acid mediated hydrophosphonylation of heterocyclic imines: A stereoselective approach towards α -amino phosphonates. *J. Chem. Soc., Perkin Trans.* **2001**, *1*, 2804-2816.
- [43] SubbaRao, D.; Srinivasulu, D.; Rajasekhar, D.; Naga Raju, C. $\text{CeCl}_3 \cdot 7\text{H}_2\text{O} \cdot \text{SiO}_2$: Catalyst promoted microwave assisted neat synthesis, antifungal and antioxidant activities of α -diaminophosphonates. *Chin. Chem. Lett.* **2013**, *24*, 759-763.
- [44] Rajbhandari, M.; Wegner, U.; Julich, M.; Schopke, T.; Mentel, R. Screening of Nepalese medicinal plants for antiviral activity. *J. Ethnopharmacol.* **2001**, *74*, 251-255.
- [45] Anon, A. Methods for examination poultry biologics and for identifying & quantifying avian pathogens. *Nat. Acad. Sci. Washington, D. C.* **1971**.
- [46] Bauer, W.; Kirby, M. M.; Sherris, J. C.; Truck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **1966**, *45*, 493-496.
- [47] National Committee for Clinical Laboratory Standards. Methods for dilution, antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. **2000**.
- [48] Rasheed, S.; Venkataramana, K.; Chandrasekhar, K.; Fareeda, G.; NagaRaju, C. Ultrasound-assisted synthesis of novel α -aminophosphonates and their biological activity. *Arch. Pharm. Chem. Life. Sci.* **2012**, *345*, 294-301.
- [49] Murray, P. R.; Baron, E. J.; Pfaller, M. A.; Tenover, F. C.; Tenover, R. H. "Manual of Clinical Microbiology", 6th ed. Mosby Year Book, London, **1995**.

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