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Synthesis and biological activity of alkyl -2-[5-(hydroxy methyl)-5-nitro-2-oxo-1,3,2λ^5-dioxaphosphinan-2-yl]amino acid esters

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ABSTRACT

*Synthesis of a series of new alkyl -2-[5-(hydroxyl methyl)-5-nitro-2-oxo-1, 3, 2λ^5-dioxaphosphinan-2-yl] amino acid esters (**5a-j**) was accomplished through a two-step process. The key step in the synthesis of **5a-j** involves preparation of a dichloride intermediate (**3a-j**), with different aminoacid esters and with a few substituted phenols by reacting $POCl_3$ in presence of Et_3N in THF at 0-15 ^oC. In the second step the intermediate in situ is treated with tris (hydroxyl methyl) nitromethane (2-hydroxy methyl-2-nitro-1, 3-propanediol) (**4**) in the presence of Et_3N at 40-45^oC to form **5a-j**. The structures of **5a-j** were established by elemental analysis, IR, ^1H, ^{13}C and ^{31}P NMR and mass spectral data. The antimicrobial activity of these compounds was evaluated and they exhibited moderate antifungal and anti bacterial activities.*

Key words: Amino acid esters, Tris(hydroxy methyl) nitromethane, 1, 3, 2-dioxaphosphinan-2-oxides, antimicrobial activity.

INTRODUCTION

1,3,2-Dioxaphosphorinane derivatives are an important class of organophosphorus compounds, which continue to attract considerable interest due to their unique features , ubiquitous in biological systems [1] and have been found multifaceted applications as important pharmacophores in agriculture [2], pharmaceutical chemistry [3] , chemical synthetic agents [4] and diverse potential biological importance [5, 6]. A few of the phosphorus compounds containing phosphinan group are eco-friendly because they degrade hydrolytically and enzymatically to non-toxic residues, so they represent an important class of insecticidal,

pesticidal, antitumour and antiviral agents [7, 8]. The phosphoryl group is of fundamental significance in many of the most important molecules that control molecular replication, cell biochemistry and metabolic processes in all living species [9]. α-Amino phosphoryl, α-hydroxy phosphonate compounds have recently been proved to be biologically active [10] and have been shown to inhibit the enzymes renin, EPSP synthase and HIV protease [11]. The 1, 3, 2-dioxaphosphinane ring linked to unsaturated α-hydroxyphosphonates were used as plant growth regulators [12]. The attachment of an amino acid group to the phosphate moiety is expected to increase their cellular uptake and thus enhances their chemotherapeutic properties. Phosphorus compounds containing an esterified amino acid group on the phosphorus atom have been found to display useful anti-neo-plastic properties [13, 14]. This back ground, and clinical success of cyclophosphamide as an anti-cancer drug [15], prompted us for the synthesis of new 6-membered phosphorus heterocycles in which aminoacid esters are linked to the phosphorus atom with potential bio-activity.

EXPERIMENTAL SECTION

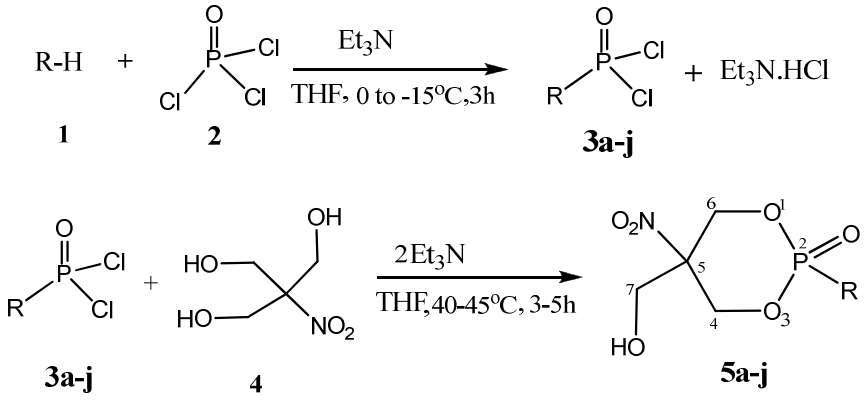
All the chemicals were purchased from Aldrich and used without further purification. TLC was performed on precoated plates with silica gel $60F_{254}$ (Merk). Column chromatography was performed on silica gel (0.040-.063 mm, Macherey Nagel). Melting points were recorded on Buchi R-535 (Flawil, Switzerland) apparatus and are uncorrected. IR Spectra were recorded on JASCO Japan FT/IR -5300 Spectrophotometer at University of Hyderabad, Hyderabad using KBr optics. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on Bruker A VIII 500 MHz NMR spectrometer at IIT, Chennai, operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C and 202 MHz for ^{31}P. NMR data were recorded in DMSO-d_6 and were referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P). Mass spectra were recorded on JEOL GCmate at IIT, Chennai and on LCMS-2010A Shimadzu, Japan, spectrometer at University of Hyderabad, Hyderabad. Elementary analyses were performed using EA 1112 Thermo Finnigan, France, instrument at University of Hyderabad, Hyderabad, India.

Preparation of Intermediate: ethyl [(dichlorophosphoryl)amino](phenyl) acetate (3a).

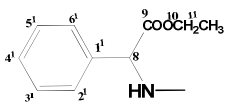
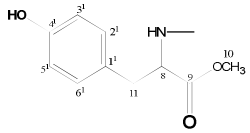
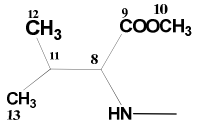
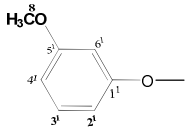
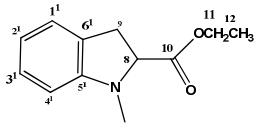
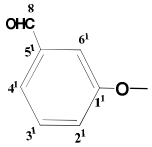
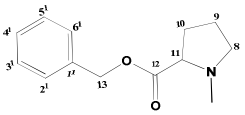
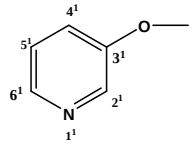
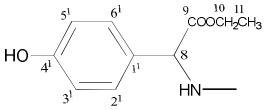
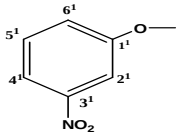
A solution of $POCl_3$ (0.28 mL, 0.003 mole) in 20 mL of dry THF was added dropwise over a period of 20 min to a stirred solution of phenyl glycine ethyl ester (**1**) (0.003 mole) and triethyl amine (0.9 mL, 0.003 mole) in 25 mL of THF at 0 to -15 ^{o}C. After stirring for 3h at 30-40^{o}C, formation of the intermediate, ethyl [(dichlorophosphoryl)amino](phenyl)acetate (**3a**) was ascertained by TLC analysis, run in a 3:7 mixture of ethylacetate and hexane and the average of R_f value observed was 0.75 . Triethylamine hydrochloride was removed by filtration. The filtrate was used for the next reaction step without further purification.

Typical Procedure for the Synthesis of 5a-j

A solution of the intermediate (**3**) (0.003 mole) in dry THF was added dropwise to a solution of tris (hydroxy methyl) nitro methane (**4**) (0.453 g, 0.003 mole) and triethyl amine (1.8 mL, 0.006 mole) in dry THF (20 mL) at 0^{o}C. After the completion of the addition, the temperature of the reaction mixture was raised to 40-45 ^{o}C and the reaction mixture was stirred for 3-5 h. After the completion of the reaction, as indicated by TLC conducted in 3:7 mixture of ethyl acetate and hexane, an average R_f value of 0.60 was observed. The reaction mixture was filtered to remove solid triethyl amine hydrochloride and the solvent was removed in a rotaevaporator to get the crude product. It was purified by column chromatography on silica gel (100-200 mesh) using ethylacetate:hexane (1:9) as eluent to afford the pure compound. The compounds thus obtained were characterised by IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data.



Scheme 1

Compound	R	Compound	R
5a		**5f**	
5b		**5g**	
5c		**5h**	
5d		**5i**	
5e		**5j**	

Spectral Data

***Ethyl {[5-(hydroxymethyl)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-2-yl]amino}(phenyl)acetate* (5a).**

Yield 70%, solid, m. p 145-147^{o}C, υ_{max} (KBr), 3396 (-OH), 3313 (P-NH), 1655 (C=O), 1545 (NO_2), 1256 (P=O), 1072 (P-O-$C_{aliphatic}$); δ_H (500 MHz, DMSO-d_6), 7.24-7.40 (5H, m, Ar-H), 4.01-4.39 (m, 4H, 4 & 6 –CH_2) , 8.63 (1H, d, J =10 Hz, P(O)-NH-C), 3.75 (2H, s, -CH_2-OH), 5.09 (1H, s, -CH_2-OH), 4.48-4.59 (1H, m,-N-CH(Ph)-C) , 2.98-3.02 (2H, q,-O-CH_2CH_3), 1.15-1.25 (3H, t, J = 12 Hz, -O-CH_2CH_3) ; δ_C (125 MHz, DMSO-d_6), 70.1 (C-4 & C-6), 95.7 (C-5), 45.9 (C-7), 58.8 (C-8), 179.7 (C=O),59. 5 (C-10), 9.1 (C-11), 129.4 (C-1^1), 129.0 (C-2^1& C-6^1), 128.8 (C-3^1&C-5^1), 127.2 (C-4^1); δ_P (202.44 MHz, 85%, H_3PO_4), -10.10 ppm; *m/z* (LCMS) 375 $(M)^+$; Anal. Calc. for $C_{14}H_{19}N_2O_8P$: C, 44.93; H, 5.12; N, 7.48%; found: C, 44.92; H, 5.20; N, 7.41%.

***Methyl 2-{[5-(hydroxymethyl)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-2-yl]amino}-3-methylbutanoate* (5b).**

Yield 65% , solid, m. p 133-135^{o}C, υ_{max} (KBr), 3360 (-OH), 3325 (P-NH), 1633 (C=O), 1547 (NO_2), 1252 (P=O), 1035 (P-O-$C_{aliphatic}$); δ_H (500 MHz, DMSO-d_6), 3.59-3.64 (m, 4H, 4 & 6 –CH_2), 8.17 (1H, d, J =8 Hz, P(O)-NH-C), 3.69 (2H, s, -CH_2-OH), 4.90 (1H, s, -OH), 0.96-0.98 (6H, d , J = 10 Hz, -CH(CH_3)$_2$), 0.83-0.87 (1H, m, -CH(CH_3)$_2$) , 3.74 (3H, s, -O-CH_3), 3.34-3.38 (1H, t, J =10 Hz, N-CH-C); δ_C (125 MHz, DMSO-d_6), 68.7 (C-4 & C-6), 95.1 (C-5), 45.5 (C-7),52.5 (C-8), 171.6 (C=O), 52.4 (C-10), 32.3 (C-11), 18.7 (C-12 & 13); δ_P (202 MHz, 85%, H_3PO_4), -11.82 ppm; *m/z* (LCMS) 327 (M^+); Anal.Calc. for $C_{10}H_{19}N_2O_8P$: C, 36.82; H, 5.87; N, 8.29%; found: C, 36.91; H, 5.90; N, 8.31%.

***Ethyl 1-[5-(hydroxymethyl)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-2-yl]-2,3-dihydro-1H-indole-2-carboxylate* (5c).**

Yield 67%, solid, m.p 153-155^{o}C; υ_{max} (KBr), 3391 (OH), 1645 (C=O),1541 (NO_2), 1257 (P=O), 1033 (P-O-$C_{aliphatic}$); δ_H (500 MHz , DMSO-d_6), 7.26-7.68 (4H,m, Ar-H), 5.00-5.28 (m, 4H, 4 & 6 –CH_2), 3.51 (2H, s, -CH_2-OH), 4.98 (1H, s, -OH), 3.82 -3.85 (1H, t. J = 10 Hz, N-CH-CH_2), 2.93-2.96 (2H, dd, CH-CH_2-), 4.02-4.52 (2H, q, O-CH_2CH_3), 1.60-1.84 (3H, t, J = 6.6 Hz, O-CH_2CH_3); δ_C (125 MHz, DMSO-d_6), 63.7 (C-4 & C-6), 94.9 (C-5), 46.1 (C-7), 40.2 (C-8), 39.4 (C-9), 171.7 (C=O), 60.6 (C-11), 14.0 (C-12), 109.0 (1^1), 126.0 (2^1), 119.1 (3^1), 126.1 (4^1), 128.1 (5^1), 144.9 (6^1); δ_P (202 MHz, H_3PO_4) -12.02 ppm; *m/z* (LCMS) 387 $(M)^+$ Anal.Calc. for $C_{15}H_{19}N_2O_8P$: C, 46.64; H, 4.96; N, 7.25%; found: C, 46.59; H, 5.07; N, 7.43%.

Benzyl 1-[5-(hydroxymethyl)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-2-yl]pyrrolidine-2-carboxylate (5d).

Yield 63% ,solid, m.p. 148-150^{o}C; υ_{max} (KBr), 3368(-OH), 1635(C=O), 1541 (NO_2), 1263 (P=O), 1037 (P-O-$C_{aliphatic}$); δ_H (500 MHz, DMSO-d_6); 7.36-7.39 (5H, m, Ar-H), 3.97-4.17 (m, 4H, 4 & 6 –CH_2), 3.74 (2H, s, -CH_2-OH), 5.52 (1H, s, -OH), 3.35-3.34 (2H, m, N-CH_2-), 1.73-1.98 (2H, m, N-CH_2-CH_2-), 2.49-2.51 (1H, m, N-CH_2-CH_2-CH_2-), 3.74 (1H, s, N-CH-), 5.11-5.17 (2H, t, J = 11 Hz, CH_2-Ph),; δ_C (125 MHz, DMSO-d_6), 67.9 (C-4 & C-6), 94.9 (C-5), 58.7 (C-7), 40.7 (C-8), 25.1 (C-9), 30.5 (C-10), 50.1 (C-11), 173.1 (C=O), 60.2 (C-13), 135.9 (1^1), 126.9 (2^1& 6^1), 130.1 (3^1 &5^1), 128.5 (4^1); δ_P (202 MHz, H_3PO_4) -13.54 ppm; *m/z* (LCMS); 401 $(M)^+$; Anal. Calc. for $C_{16}H_{21}N_2O_8P$: C, 48.00; H, 5.29; N, 7.00%; found: C, 48.03; H, 5.31; N, 7.16%.

Ethyl {[5-(hydroxymethyl)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-2-yl]amino}(4-hydroxyphenyl) acetate (5e).

Yield 60% solid, m.p. 149-151^{o}C; υ_{max} (KBr), 3379 (-OH), 3323 (P-NH), 1612 (C=O), 1539 (NO_2), 1290 (P=O), 1035 (P-O-$C_{aliphatic}$); δ_H (500 MHz, DMSO-d_6), 7.26-7.41 (4H, m, Ar-H), 3.97-4.17 (m, 4H, 4 & 6 –CH_2), 3.75 (2H, s, -CH_2-OH), 5.51 (1H, s, -OH), 8.10 (1H, d, J =8 Hz, P(O)-NH-C), 9.54 (1H, s, Ar-OH), 3.58-3.62 (2H, q,-O-CH_2CH_3), 1.25-1.29 (3H, t, J =10 Hz, -O-CH_2CH_3); δ_C (125 MHz, DMSO-d_6), 69.6 (C-4 & C-6), 89.7 (C-5), 50.8 (C-7), 50.2 (C-8), 170.3 (C=O), 60.1(C-10), 15.3 (C-11), 130.1 (1^1), 131.1 (2^1 & 6^1), 115.3 (3^1 & 5^1), 160.1 (4^1); δ_P (202 MHz, 85%H_3PO_4), -9.65 ppm; *m/z* (LCMS) 391 $(M)^+$; Anal. Calc. for $C_{16}H_{21}N_2O_8P$: C, 43.08; H, 4.91; N, 7.18%; found: C, 42.93; H, 5.01; N, 7.16%.

Methyl2-{[5-(hydroxymethyl)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-2-yl]amino}-3-(4-hydroxy phenyl)propanoate (5f).

Yield 69 % solid, m.p. 139-141^{o}C; υ_{max} (KBr), 3373 (-OH), 3326 (P-NH), 1605 (C=O), 1535 (NO_2), 1292 (P=O), 1010 (P-O-$C_{aliphatic}$); δ_H (500 MHz, DMSO-d_6), 7.84-7.88 (4H, m, Ar-H), 3.75-3.98 (m, 4H, 4 & 6 –CH_2), 3.65 (2H, s, -CH_2-OH), 5.01 (1H, s, -OH), 8.43 (1H, d, J =10 Hz, P(O)-NH-C), 10.15 (1H, s, Ar-OH), 3.04 (1H, s,-O-CH_3), 3.39-3.44 (1H, t, J =15Hz, N-

CH-), 3.04, 3.16 (2H, s, -CH_2-Ph); δ_C (125 MHz, DMSO-*d_6*), 67.8 (C-4 & C-6), 90.7 (C-5), 54.8 (C-7), 50.1 (C-8), 174.9 (C=O), 50.1(C-10), 39.7 (C-11), 129.3 (1^1), 130.3 (2^1 & 6^1), 115.5 (3^1 & 5^1), 155.8 (4^1); δ_P (202 MHz, 85% H_3PO_4) -10.20 ppm; *m/z* (LCMS) 391 $(M)^+$; Anal. Calc. for $C_{14}H_{19}N_2O_9P$: C, 43.08; H, 4.91; N, 7.18%; found: C, 43.11; H, 4.95; N, 7.36%.

[2-(3-Methoxyphenoxy)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-5-yl]methanol (5g)

Yield 59 % solid, m.p. 132-134^{o}C; υ_{max} (KBr), 3393 (-OH), 3306 (P-NH), 1528 (NO_2), 1286 (P=O), 1018 (P-O-$C_{aliphatic}$), 945 (P-O-($C_{aromatic}$)), 1235 ((P)-O-$C_{aromatic}$); δ_H (500 MHz, DMSO-*d_6*), 7.21-7.35 (4H, m, Ar-H), 4.32-4.71 (m, 4H, 4 & 6 –CH_2), 3.94 (2H, s, -CH_2-OH), 5.12 (1H, s, -OH), 3.50 (3H, s, Ar-O-CH_3), δ_C (125 MHz, DMSO-*d_6*), 68.6 (C-4 & C-6), 90.7 (C-5), 54.8 (C-7), 55.2 (C-8), 150.1 (1^1), 111.1 (2^1), 135.3 (3^1), 106.1 (4^1), 161.0 (5^1), 103.1 (6^1); δ_P (202 MHz, 85%H_3PO_4) -12.20 ppm; *m/z* (LCMS) 320 $(M)^+$; Anal. Calc. for $C_{11}H_{14}NO_8P$: C, 41.39; H,4.42; N, 4.39%; found: C, 41.18; H, 4.25; N, 4.36%.

3-{[5-(Hydroxymethyl)-5-nitro-2-oxido-1,3,2-dioxaphosphinan-2-yl]oxy}benzaldehyde (5h).

Yield 68 % solid, m.p. 109-111^{o}C; υ_{max} (KBr), 3396 (-OH), 3353 (P-NH), 1543 (NO_2), ,1303 (P=O), 1005 (P-O-$C_{aliphatic}$), 962 (P-O-($C_{aromatic}$)), 1228 ((P)-O-$C_{aromatic}$); δ_H (500 MHz, DMSO-*d_6*), 7.67-7.73 (4H, m, Ar-H), 4.12-4.15 (m, 4H, 4 & 6 –CH_2), 3.76 (2H, s, -CH_2-OH), 5.32 (1H, s, -OH), 9.91 (Ar-CHO); δ_C (125 MHz, DMSO-*d_6*), 66.6 (C-4 & C-6), 88.7 (C-5), 54.8(C-7), 191.5 (C-8), 150.1 (1^1), 127.1 (2^1), 132.3 (3^1) 120.5 (4^1), 138.5 (5^1), 115.9 (6^1); δ_P (202 MHz, 85%H_3PO_4), -13.82 ppm; *m/z* (LCMS) 318 $(M+H)^{+\cdot}$; Anal. Calc. for $C_{11}H_{12}NO_8P$: C, 41.65; H, 3.81; N, 4.42%; found: C, 41.73; H, 3.85; N, 4.46%.

[5-Nitro-2-oxido-2-(pyridin-2-yloxy)-1,3,2-dioxaphosphinan-5-yl]methanol (5i).

Yield 59 % solid, m.p. 132-134^{o}C; υ_{max} (KBr), 3387 (-OH), 3295 (P-NH), 1541 (NO_2), ,1296 (P=O), 1024 (P-O-$C_{aliphatic}$), 935 (P-O-($C_{aromatic}$)), 1236 ((P)-O-$C_{aromatic}$); δ_H (500 MHz DMSO-*d_6*), 6.67-7.23 (4H, m, Ar-H), 4.02-4.10 (m, 4H, 4 & 6 –CH_2), 3.96 (2H, s, -CH_2-OH), 5.12 (1H, s, -OH); δ_C (125 MHz, DMSO-*d_6*), 68.1 (C-4 & C-6), 89.0 (C-5), 54.9 (C-7), 114.1 (1^1), 115.3 (2^1), 135.1 (3^1), 110.1 (4^1), 141.0 (5^1); δ_P (202 MHz, 85%H_3PO_4), -14.20 ppm; *m/z* (LCMS) 291 $(M+H)^{+\cdot}$; Anal. Calc. for $C_9H_{11}N_2O_7P$: C, 37.25; H, 3.82; N, 9.65%; found: C, 37.07; H, 3.80; N, 9.62%.

Table1. Antifungal activity of compounds 5a-j

Compound	Zone of inhibition/mm			
	Colletotrichum gloeosporioides		*Aspergillus niger*	
	250[b]	500	250	500
5a	14	19	15	21
5b	12	16	12	18
5c	8	11	7	12
5d	6	9	8	17
5e	12	15	10	15
5f	10	16	8	13
5g	8	14	9	14
5h	7	9	11	18
5i	10	14	9	16
5j	11	18	8	14
Griseofulvin[a]	20		21	

[a]*Reference compound*
[b]*Concentration in µg/disc*

[5-Nitro-2-(3-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphinan-5-yl]methanol (5j).

Yield 65 % solid, m.p. 106-108^{o}C; υ_{max} (KBr), 3357 (-OH), 3298 (P-NH), 1531 (NO_2), 1301 (P=O), 1074 (P-O-$C_{aliphatic}$), 960 (P-O-($C_{aromatic}$)), 1196 ((P)-O-$C_{aromatic}$); δ_H (500 MHz DMSO-

d$_6$), 7.60-7.76 (4H, m, Ar-H), 3.97-4.07 (m, 4H, 4 & 6 –CH_2), 3.84 (2H, s, -$\underline{CH_2}$-OH), 5.01 (1H, s, -OH); δ_C (125 MHz, DMSO-*d*$_6$), 68.1 (C-4 & C-6), 90.7 (C-5), 54.8(C-7), 150.1 (1^1), 126.1 (2^1), 131.3 (3^1), 116.1 (4^1), 149.2 (5^1), 110.1 (6^1); δ_P (202 MHz, 85% H_3PO_4) -11.20 ppm; *m/z* (LCMS) 335 $(M+H)^{+\cdot}$; Anal. Calc. for $C_{10}H_{11}N_2O_9P$: C, 35.94, H, 3.32, N, 8.38%; found: C, 35.89; H, 3.30; N, 8.31%.

Table2. Antibacterial activity of compounds 5a-j

Compound	Zone of inhibition/mm[a]							
	Staphylococcus aureus		*Bacillus subtilis*		*Escherichia coli*		*Klebsiella pneumoniae*	
	250[c]	500	250	500	250	500	250	500
5a	8	17	9	12	8	10	10	16
5b	9	15	10	15	6	11	8	14
5c	8	14	6	13	9	14	9	13
5d	6	12	9	17	10	12	7	11
5e	11	20	7	10	7	10	9	13
5f	-	8	8	15	-	7	9	12
5g	7	14	6	13	9	17	6	15
5h	10	15	11	16	13	16	11	14
5i	7	12	9	14	9	13	8	14
5j	11	16	13	15	8	15	-	9
Penicillin[b]	20		20		20		20	

[a]*-Indicates no activity*
[b]*Reference compound*
[c]*Concentration in µg/disc*

RESULTS AND DISCUSSION

The synthesis of novel alkyl -2-[5-(hydroxy methyl)-5-nitro-2-oxo-1,3,2λ^5-dioxaphosphinan-2-yl] amino acid esters (**5a-j**) is accomplished in a two-step process. The synthetic route involves the reaction of various aminoacid esters (**1**) with $POCl_3$ (phosphorus oxy chloride) in dry THF in presence of triethyl amine at -15^{o}C to afford the corresponding intermediate dichlorides (**3a-j**). In the second step the intermediate **3** was reacted with tris(hydroxy methyl) nitromethane (**4**) in dry THF in presence of triethylamine to afford the title compounds (**5a-j**) in good yields (**Scheme 1**). The second step of the reaction was completed at 40-45^{o}C with stirring for 3-5 hours. The progress of the reaction was monitored by TLC analysis at different time intervals and the crude products obtained after removing the solvent were purified by column chromatography on silica gel using ethylacetate and hexane (4:6) as step grade mixtures as eluents. The synthetic and analytical data of title compounds (**5a-j**) are given in the experimental part. All the compounds (**5a-j**) exhibited absorption bands for -OH, P-NH, P=O, NO_2, C=O and P-O-C $_{aliphatic}$ in the regions 3357-3396, 3295-3353, 1252-1303, 1528-1547, 1602-1655, and 1010-1124 cm^{-1} respectively [16, 17]. P-O-C$_{aromatic}$ for a few compounds (**5g-j**) gave two absorptions in the regions 935-960 and 1196-1236 cm^{-1}. The ^{1}H NMR spectra (500 MHz) of **5a-j** exhibited multiplets in the range of δ 4.03-4.08 accounting for the 4 and 6 methylene protons of the dioxaphosphinan [18]. The aromatic protons of **5a-j** resonated as multiplets at δ 6.67-7.76. The –NH proton signal was observed at δ 8.10-8.63 as a doublet (*J*=8-10 Hz). The –CH_2-$\underline{OH}$ protons of **5a-j** resonated at δ 4.90-5.53 as a singlet. The –$\underline{CH_2}$-OH protons of **5a-j** resonated at δ 3.51-3.96 as a singlet. The remaining protons gave signals in the expected regions. The ^{13}C NMR spectral data of all the compounds were recorded and the data are given in the experimental part. The C-4 and C-6 carbons resonated at δ 63.7-70.1, C-5 signal appeared at δ 89.7-95.7 and C-7 signal observed at δ 45.5-54.8. The C=O carbon gave signal at δ 170.3-179.7. The remaining carbon signals are observed in the expected regions [19]. Compounds **5a-j** exhibited phosphorus-

31 resonance signals in the range of -14.20 to -9.65 ppm [20]. The GC-MS and LC-MS of a few of the compounds were recorded and the presence of M^+, $(M-CH_3)^+$, $(M-2CH_3)^+$,$(M-C_2H_7)^+$,$(M-C_3H_7)^+$ ·, $(M-C_4H_9)^+$,(M-OR)$^+$ ions in their mass spectra indicate that they undergo a similar fragmentation pattern and the data are presented in the experimental section.

Antimicrobial activity

Susceptibility of test organisms to the title compounds (**5a-j**) was determined by employing the standard disc diffusion technique [21]. All the compounds (**5a-j**) were tested for their antifungal activity against the growth of *Colletotrichum gleosorioides and Aspergillus niger* along with the standard fungicide Griseofulvin at concentrations of 250 and 500 µg/disc, according to the procedure of Horsfall and Rich [22] (**Table1**). Compounds **5a-j** were also screened for their anti bacterial activity against the growth of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella peumoniae* along with the standard Penicillin at concentrations of 250 and 500 µg/disc according to the method of Vincent and Vincent [23] (**Table2**). The results revealed that these compounds exhibited moderate anti fungal and anti bacterial activity against the tested species.

CONCLUSION

A new class of alkyl -2-[5-(hydroxy methyl)-5-nitro-2-oxo-1,3,2λ^5-dioxaphosphinan-2-yl] amino acid esters with moderate antimicrobial activity were conveniently synthesized in good yields.

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