Design, Synthesis and Anti Colon Cancer Activity Evaluation of Phosphorylated Derivatives of Lamivudine (3TC)

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Abstract: The novel phosphorylated derivatives of Lamivudine (**5a-5l**) as potential anti colon cancer agents are synthesized. These title compounds are designed based on the basic pyrimidine derivative lamivudine as a starting compound and reacted with various phosphorodichloridates followed by the introduction of bioactive groups at the phosphorus. Their structures were characterized by IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral analyses. All the compounds were evaluated for their anti colon cancer activity against COLO-205 cell lines *in vitro* studies. Among them **5a** and **5b** emerged as lead compounds with 0.003 μM and 0.0001 μM values.

Keywords: Anti colon cancer activity, COLO- 205 cell lines, MTT assay, Phosphorylated derivatives of lamivudine.

INTRODUCTION

Nucleoside analogs, such as lamivudine, zidovudine, zalcitabine and didanosine are an important class of anticancer and antiviral agents [1, 2]. Lamivudine (3TC), the negative enatiomer of (-) 2-deoxy-3′-thiacytidine is a dideoxy nucleoside which was disclosed by *Soudeyns et al.* a structurally novel potent anti-HIV analog BCH -189 (2'-3'-dideoxy-3'-thiacytidine) [3], in which the methylene group at the 3′-position of the ribose ring is replaced by a sulfur atom. Lamivudine is a dideoxynucleoside analog reverse transcriptase inhibitor that is used in combination with other nucleoside reverse transcriptase inhibitor, non-nucleoside reverse transcriptase inhibitor, and protease inhibitors in the treatment of HIV-1 infection as a component of currently recommended highly active antiretroviral therapy [4]. Among D-dideoxynucleoside analogs, 2′-3′ didehydro-2′, 3′ dideoxythymidine and ddC (2'-3'-dideoxycytidine) are a few examples of anti-HIV drugs and D-dideoxynucleoside analogs like β-D- arabinofuranosylcytosine and gemcitabine are anticancer drugs [5].

Like other dideoxynucleosides, the antiviral activity of lamivudine is due to its active 5′-triphosphate anabolite [6]. Lamivudine is anabolized intracellularly by a step wise process, first to the monophosphate form by deoxycytidine kinase, then to the diphosphate form by cytidine monophosphate kinase, and deoxycytidine monophosphate kinase, and finally to the active triphosphate form by pyrimidine nucleoside diphosphate kinase [6]. *In vitro* studies have shown lamivudine- diphosphate to be the predominant anabolite; thus, the rate-limiting step in lamivudine phosphorylation is from lamivudine- diphosphate to lamivudine- triphosphate [7]. To exert its antiretroviral effect, lamivudine- triphosphate competes with endogenous 2′-deoxycytidine–5′-triphosphate for binding on HIV-I reverse transcriptase. As lamivudine- triphosphate does not have a hydroxyl group at any position analogous to the 3′- hydroxyl group of the natural 2′-deoxy nucleosides, its use as a substrate by HIV-I reverse transcriptase effectively terminates chain extension beyond its incorporation into the HIV-DNA genome [8].

Lamivudine has been proved in clinical use for the treatment of HIV and hepatitis-B [9], L-FMAU [1-(2-fluoro-5-methyl-beta,L-arabinofuranosyl) uracil] [10] and 2′, 3′-dideoxy-2′, 3′-didehydro-β-L-5′-fluorodeoxycytidine, which are in phase II clinical trials as anti hepatitis-B virus agents, and β-L (-)-dioxolanecytidine [11, 12] which is in the phase II clinical trials as an anti cancer agent. This compound is in the advanced stage of clinical evaluation for the treatment of both diseases. Clinical trials also demonstrated that 3'-azido-3'-deoxythymidine has a significant effect in controlling the progression of AIDS in patients [13]. Critical clinical evaluation of the therapeutic potential of nucleoside analogs requires a large amount of material be readily accessible which translates into efficient synthetic protocols for preparation of derivatives with bio-active groups.

Lamivudine is the derivative of pyrimidine, and although originally developed as an antiviral for human immuno deficiency virus (HIV) was found to selectively repress COLO-205 cell line and is also effective for repression of necroinflammation, liver fibrosis, seroconversion of serum HBV-DNA and HBeAg (hepatitis B "e" antigen), and normalization of serum ALT (alanine amino transferase) [14]. Further, pharmacokinetic properties and cellular permeability of a drug can be modulated by derivatization of bioreversible forms of this drug.

The current work describes such an effort in which phosphorylated derivatives of lamivudine are synthesized and screening for anti colon cancer activity against COLO -205

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cell lines in *in vitro* was envisaged. The anti-cancer activity of D-deoxy nucleoside analongs led us to develop the synthesis of new phosphorylated derivatives of lamivudine. Substituents were selected with different electronic and solubility characteristics with the aim of investigating the substituent effect at the phosphorus atom.

MATERIALS AND METHODS

Chemicals were procured from Sigma-Aldrich, Merck and Lancaster, and 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 [15]. Melting points (m.p.) were determined using a calibrated thermometer by Guna Digital Melting Point apparatus. They are expressed in degrees centigrade (°C) and are uncorrected. Infrared Spectra (IR) were obtained on a Nicolet 380 FT-IR spectrophotometer. Samples were analyzed as potassium bromide (KBr) discs. Absorptions are reported in wave numbers (cm^{-1}). ^{1}H, ^{13}C NMR and P^{31}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 NMR. The ^{1}H and ^{13}C chemical shifts are expressed in parts per million (ppm) with reference to tetramethylsilane (TMS) and ^{31}P chemical shifts to 85% H_3PO_4. LC mass spectra were recorded on a Jeol SX 102 DA / 600 Mass spectrometer. Elemental analyses were performed by Central Drug Research Institute, Lucknow, INDIA. The following abbreviations were used while presenting the NMR data s = singlet, d = doublet, t = triplet and m = multiplet.

SYNTHESIS

To the stirred solution of lamivudine (228.8 mg, 1.00 mmol) in dry tetrahydrofuran (20 mL) and pyridine (20 mL), was added a solution of 4-nitrophenyl phosphorodichloridate (137.8 mg, 1.00 mmol) in THF (20 mL) at -10 ºC in the presence of TEA (101.2 mg, 1.00 mmol) over a period of 15 min. The reaction mixture was further stirred at 0 ºC and continued stirring for 2 h, progress of the reaction was monitored by TLC, (ethylacetate: hexane 5:5). After completion of the reaction, it was filtered to remove triethylamine hydrochloride. The filtrate containing the intermediate **3a** was further reacted with mono potassium dihydrogen phosphate (136.0 mg, 1.00 mmol) in THF (10 mL) and pyridine (10 mL) at 70-80 ºC for 10 hrs. After completion of the reaction, NaCl was removed by filtration and the solvent was removed in a rota-evaporator to obtain crude product. It was purified by column chromatography on silica gel using acetone: methanol (9:1) as eluent to afford the pure compound **5a,** [5-(4-amino-2-oxo-1, 2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl]methyl(2-dihydrogenphosphato) (4-nitrophenyl)phosphate. The same experimental procedure was adopted for the preparation of the remaining title compounds **5b-5l**. Analytical and spectral data of the newly synthesized compounds corroborated well with the proposed structures. The synthetic protocol for all the compounds **5a–5l** is presented in Scheme **1** and their spectral data are given below.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl]methyl(2-dihydrogenphosphato)(4-nitrophenyl) phosphate (5a)

Yield 72%; m.p. 171-173 ºC; IR (ν_{max}, cm^{-1}, KBr): 3415 (O-H), 3295 (N-H), 1228 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6 , δ , ppm): 3.10 (dd, 1H, *J* = 4.1, 11.9 Hz), 3.43 (dd, 1H, *J* = 5.2, 11.5 Hz), 3.76-3.80 (m, 2H), 5.19-5.21 (m, 1H), 5.80 (d, 1H, *J* = 7.6 Hz), 6.20-6.23 (m, lH), 7.30-7.42 (m, 2H), 7.72-7.92 (br m, 2H), 7.98 (d,1H, *J* = 7.6 Hz), 8.10-8.22 (m, 2H), 9.6 (br s, 2H, P-OH); ^{13}C NMR (100 MHz, DMSO-d_6 , δ , ppm): 38.5, 67.4, 86.9, 87.5, 96.5, 120.6, 125.6, 141.1, 142.2, 152.8, 159.6, 164.7; ^{31}P (161.9 MHz, DMSO-d_6 , δ , ppm): -7.16, -21.30, LCMS (m/z,%): 510 [$MH^{+\bullet}$, 100], 359 (28), 344 (31), 285 (19); Anal. Calcd.: $C_{14}H_{16}N_4O_{11}P_2S$: C 32.95, H 3.16, N 10.98; Found: C 32.62, H 3.14, N 10.95.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl]methyl(2-hydroxyethyloxy)(4-nitrophenyl)phosphate (5b)

Yield: 75%; m.p.:126-128 ºC; IR (ν_{max}, cm^{-1}, KBr): 3403 (O-H), 3278 (N-H), 1235 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6 , δ, ppm): 3.14 (dd, 1H, *J* = 4.2, 11.2 Hz), 3.44 (dd, 1H, *J* = 5.2 , 11.2 Hz), 3.78-3.84 (m, 2H), 4.10-4.14 (m, 2H), 4.30-4.33 (m, 1H), 4.52 (t, 1H, *J* = 5.2 Hz), 5.16-5.20 (m, 1H), 5.80 (d, 1H, *J* = 7.6 Hz), 6.22-6.28 (m, 1H), 7.35-7.48 (m, 2H), 7.42 (d, 1H, *J* = 5.3 Hz), 7.81-7.86 (br m, 2H), 7.92 (d, 1H, *J* = 7.5 Hz), 8.23-8.35 (m, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6 , δ , ppm): 37.6, 62.3, 67.6, 68.8, 75.2, 84.5, 96.3, 121.2, 125.3, 141.3, 143.2, 152.5, 159.2, 164.5; ^{31}P (161.9 MHz, DMSO-d_6 , δ , ppm): -4.50; LCMS (m/z,%): 474 [$MH^{+\bullet}$, 27], 387 (15), 314 (20), 277 (48), 197 (10), 183 (30), 100 (41); Anal. Calcd.: $C_{16}H_{19}N_4O_9PS$: C 40.51, H 4.04, N 11.81; Found: C 40.30, H 3.99, N 11.72.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methyl(2-aminoethylamido) (4-nitrophenyl) phosphate (5c)

Yield: 69%; m.p.: 182-184 ºC; IR (ν_{max}, cm^{-1}, KBr): 3292 (N-H), 1229 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6 , δ , ppm): 3.11 (dd, 1H, *J* = 4.2, 11.5 Hz), 3.40 (dd, 1H, *J* = 5.2 Hz, 5.4 Hz), 3.56-3.61 (m, 4H), 3.80-3.85 (m, 2H), 4.34-4.42 (m, 1H), 5.19-5.21 (m, 1H), 5.26 (br s, 2H), 5.80 (d, 1H, *J* = 7.4 Hz), 6.31-6.38 (m, 1H), 7.28-7.37 (m, 2H), 7.82-7.88 (br m, 2H), 7.96 (d, 1H, *J* = 7.4 Hz), 8. 10-8.16 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6 , δ , ppm): 38.2, 40.2, 45.1, 3, 67.2, 86.5, 87.5, 95.5, 121.7, 126.3, 142.2, 143.5, 152.3, 158.4, 164.5; ^{31}P (161.9 MHz, DMSO-d_6 , δ , ppm): -18.20; LCMS (m/z,%): 472 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{16}H_{21}N_6O_7PS$: C 40.68, H 4.48, N 17.79; Found: C 40.52, H 4.44, N 17.67.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl]methyl(4-methylpiperazino)(4-nitrophenyl) phosphate (5d)

Yield: 72%; m.p.: 116-118 ºC; IR (ν_{max}, cm^{-1}, KBr): 3302 (N-H), 1218 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6 , δ, ppm): 2.27 (s, 3H), 2.41-2.47 (m, 4H), 2.81-2.85 (m, 4H), 3.12 (dd, 1H, *J* = 4.1, 11.1 Hz), 3.43 (dd, 1H, *J* = 5.2 Hz, 4.1

Hz), 3.84-3.89 (m, 2H), 5.18-5.20 (m, 1H), 5.81 (d, 1H, *J* = 7.1 Hz), 6.21-6.26 (m, 1H), 7.22-7.38 (m, 2H), 7.88-7.94 (br m, 2H), 7.98 (d, 1H, *J* = 7.6 Hz), 8.13-8.18 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.5, 43.1, 44.1, 56.2, 67.4, 86.0, 87.2, 96.6, 120.6, 125.5, 142.6, 143.5, 152.5, 159.4, 164.1; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -7.11; LCMS (m/z,%): 512 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{19}H_{25}N_6O_7PS$: C 44.53, H 4.92, N 16.40; Found: C 44.29, H 4.87, N 16.24.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methyl (4-chlorophenyl) (2-dihydrogenphosphato)phosphate (5e)

Yield: 71%; m.p.: 126-128 ºC; IR (ν_{max}, cm^{-1}, KBr): 3408 (O-H), 3312 (N-H), 1224 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.14 (dd, 1H, *J* = 4.1, 11.4 Hz), 3.40 (dd, 1H, *J* = 5.2, 11.9 Hz), 3.80-3.85 (m, 2H), 5.20-5.24 (m, 1H), 5.80 (d, 1H, *J* = 7.4 Hz), 6.25-6.31 (m, 1H), 6.32-6.48 (m, 2H), 7.20-7.35 (m, 2H), 7.85-7.89 (br m, 2H), 7.99 (d, 1H, *J* = 7.5 Hz), 10.2 (br s, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.5, 67.4, 86.9, 87.5, 96.5, 120.6, 126.6, 128.4, 142.5, 148.3, 158.3, 164.7; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -7.40, -25.31; LCMS (m/z,%): 499 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{14}H_{16}ClN_3O_9P_2S$: C 33.65, H 3.23, N 8.41; Found.: C 33.42, H 3.20, N 8.33.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methyl (4-chlorophenyl)(2-hydroxyethyloxy) phosphate (5f)

Yield: 71%; m.p.: 126-128 ºC; IR (ν_{max}, cm^{-1}, KBr): 3408 (O-H), 3312 (N-H), 1224 (P=O); ^{1}H NMR (400 Hz, DMSO-d_6, δ, ppm): 3.10 (dd, 1H, *J* = 4.1 Hz, 11.1 Hz), 3.41 (dd, 1H, *J* = 5.2 Hz, 11.6 Hz), 3.82-3.87 (m, 2H), 4.12-4.16 (m, 2H), 4.36-4.40 (m, 1H), 5.21-5.24 (m, 1H), 5. 63 (s, 1H), 5.80 (d, 1H, *J* = 7.1 Hz), 6.20-6.24 (m, 1H), 6.17-6.23 (m, 1H), 6.35-6.46 (m, 2H), 7.22-7.30 (m, 1H), 7.87-7.92 (br m, 2H) 7.95 (d, 1H, *J* = 7.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.5, 61.2, 67.4, 68.2, 86.9, 87.5, 96.5, 120.6, 126.6, 129.5, 142.1, 148.3, 159.6, 164.7; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -7.40; LCMS (m/z,%): 463 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{16}H_{19}ClN_3O_7PS$: C 41.43, H 4.13, N 9.06; Found: C 41.12, H 4.09, N 8.79.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl]methyl(4-chlorophenyl)(2-aminoethyl amido) phosphate (5g)

Yield: 80%; m.p.: 114-116 ºC; IR (ν_{max}, cm^{-1}, KBr): 3316 (N-H), 1231 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.12 (dd, 1H, *J* = 4.1, 11.2 Hz), 3.41 (dd, 1H, J = 5.2, 11.4 Hz), 3.58-3.61 (m, 4H), 3.85-3.89 (m, 2H), 4.28-4.32 (m, 1H), 5.16-5.20 (m, 1H), 5.26 (br s, 2H), 5.80 (d, 1H, *J* = 7.5 Hz), 6.32-6.48 (m, 2H), 6.14-6.21 (m, 1H), 7.16-7.24 (m, 2H), 7.85-7.92 (br m, 2H), 7.98 (d, 1H, *J* = 7.5Hz); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.5, 40.2, 44.6, 67.4, 86.9, 87.5, 96.5, 120.6, 126.5, 130.2, 142.8, 147.7, 159.1, 164.7; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -4.10; LCMS (m/z,%): 461 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{16}H_{21}ClN_5O_5PS$: C 41.61, H 4.58, N 15.16; Found: C 41.40, H 4.54, N 15.00.

[5-(4–Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methyl(4-chlorophenyl)(4-methylpiperazino) phosphate (5h)

Yield: 74%; m.p.: 181-183 ºC; IR (ν_{max}, cm^{-1}, KBr): 3286 (N-H), 1218 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.27(s, 3H), 2.41-2.43 (m, 4H), 2.81-2.86 (m, 4H), 3.10 (dd, 1H, *J* = 4.5, 11.3 Hz), 3.42 (dd, 1H, *J* = 5.2, 11.5 Hz), 3.80-3.84(m, 2H), 4.21-4.24 (m, 1H), 5.19-5.22 (m, 1H), 5.80 (d, 1H *J* = 7.5 Hz), 6.02-6.13 (m, 2H), 6.20-6.24 (m, 1H), 7.21-7.36 (m, 2H), 7.88-7.94 (br m, 2H) 7.98 (d, 1H, *J* = 7.6 Hz), ; ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.5, 43.1, 44.1, 56.2, 67.4, 86.9, 87.5, 95.5, 120.6, 125.5, 141.1, 152.8, 142.2, 159.2, 164.7; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -7.11; LCMS (m/z,%): 501 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{19}H_{25}ClN_5O_5PS$: C 45.47, H 5.02, N 13.95; Found: C 45.26, H 4.97, N 13.82.

[5-(4-Amino-2-oxo-1, 2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methylbis(2-chloroethyl)amino(2-dihydrogenphosphato)phosphate (5i)

Yield: 71%; m.p.: 161-163 ºC; IR (ν_{max}, cm^{-1}, KBr): 3388 (O-H), 3292 (N-H), 1221 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.10 (dd, 1H, *J* = 4.5, 11.9 Hz), 3.42 (dd, 1H, *J* = 5.2, 11.5 Hz), 3.26 (t, 4H, *J* = 13.6 Hz), 3.62 (t, 4H, *J* = 13.6 Hz), 3.81-3.85 (m, 2H), 5.18-5.21 (m, 1H), 5.80 (d, 1H, *J* = 7.6 Hz), 6.20-6.24 (m, 1H), 7.86-7.95 (br m, 2H), 7.96 (d, 1H, *J* = 7.4 Hz), 11.8 (br s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.5, 43.2, 44.5, 64.2, 86.9, 87.5, 96.5, 142.2, 158.6, 164.9; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -4.11, -17.21; LCMS (m/z,%): 512 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{12}H_{20}Cl_2N_4O_8P_2S$: C 28.08, H 3.93, N 10.92; Found: C 27.86, H 3.90, N 10.82.

[5-(4-Amino-2-oxo-1, 2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methylbis(2-chloroethyl)amino(2-hydroxyethyloxy)phosphate (5j)

Yield: 72%; m.p.: 171-173 ºC; IR (ν_{max}, cm^{-1}, KBr): 3396 (O-H), 3295 (N-H), 1228 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.10 (dd, 1H, *J* = 4.5, 11.3 Hz), 3.28 (t, 4H, *J* = 13.6 Hz), 3.42 (dd, 1H, *J* = 5.2, *J* = 11.5 Hz), 3.61 (t, 4H, *J* = 13.6 Hz), 3.84-3.88 (m, 2H), 4.10-4.16 (m, 2H), 4.21-4.26 (m, 1H), 4.52 (t, 2H, *J* =13.5 Hz), 5.17-5.20 (m, 1H), 5.80 (d, 1H, *J* = 7.1 Hz), 6.18-6.24 (m, 1H), 7.85-7.92 (br m, 2H) 7.98 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 44.2, 45.5, 62.6, 67.4, 68.8, 74.5, 86.9, 87.5, 96.5, 142.2, 158.3, 165.5; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -7.11; LCMS (m/z,%): 476 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{14}H_{23}Cl_2N_4O_6PS$: C 35.23, H 4.86, N 11.74; Found: C 34.98, H 4.82, N 11.63.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methylbis (2-chloroethyl amino) (2-aminoethylamido) phosphate (5k)

Yield: 78%; m.p.: 112-114 ºC; IR (ν_{max}, cm^{-1}, KBr): 3292 (N-H), 1221 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.10 (dd, 1H, *J* = 4.1, 11.2 Hz), 3.28 (t, 4H, *J* = 13.6 Hz), 3.40 (dd, 1H, *J* = 5.2, 11.0 Hz), 3.48-3.51 (m, 4H), 3.60 (t, 4H, *J* = 13.6 Hz), 3.80-3.82 (m, 2H), 4.20-4.26 (m, 1H),

5.19-5.21 (m, 1H), 5.28 (br s, 2H), 5.80 (d, 1H, *J* = 7.2 Hz), 6.20-6.23 (m, 1H), 7.85-7.94 (br m, 2H) 7.98 (d, 1H, *J* = 7.8 Hz), ; ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 36.2, 38.5, 40.2, 45.1, 67.4, 75.1, 86.9, 87.5, 96.7, 141.2, 158.6, 166.1; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -7.11; LCMS (m/z,%): 474 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{14}H_{25}Cl_2N_6O_4PS$: C 35.38, H 5.30, N 17.68; Found: C 35.23, H 5.25, N 17.56.

[5-(4-Amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolan-2-yl] methylbis(2-chloroethyl)amino(4-methylpiperazino)phosphate (5l)

Yield: 72%; m.p.: 171-173 ºC; IR (ν_{max}, cm^{-1}, KBr): 3295 (N-H), 1228 (P=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.27 (s, 3H), 2.41-2.43 (m, 4H), 2.81-2.86 (m, 4H), 3.10 (d, 1H, *J* = 5.4, 11.2 Hz), 3.28 (t, 4H, *J* = 13.6 Hz), 3.42 (dd, 1H, *J* = 5.2 Hz, 11.5 Hz), 3.60 (t, 4H, *J* = 13.5 Hz), 3.76-3.80 (m, 2H), 5.21-5.24 (m, 1H), 5.80 (d, 1H, *J* = 7.6 Hz), 6.21-6.26 (m, 1H), 7.80-7.84 (br m, 2H), 7.99 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.5, 43.2, 44.5, 48.7, 56.8, 67.4, 75.4, 86.9, 87.5, 95.5, 141.5, 159.6, 166.5; ^{31}P (161.9 MHz, DMSO-d_6, δ, ppm): -7.11; LCMS (m/z,%): 514 [$MH^{+\bullet}$]; Anal. Calcd.: $C_{17}H_{29}Cl_2N_6O_4PS$. C 39.62, H 5.67, N 16.31; Found: C 39.42, H 5.62, N 16.15.

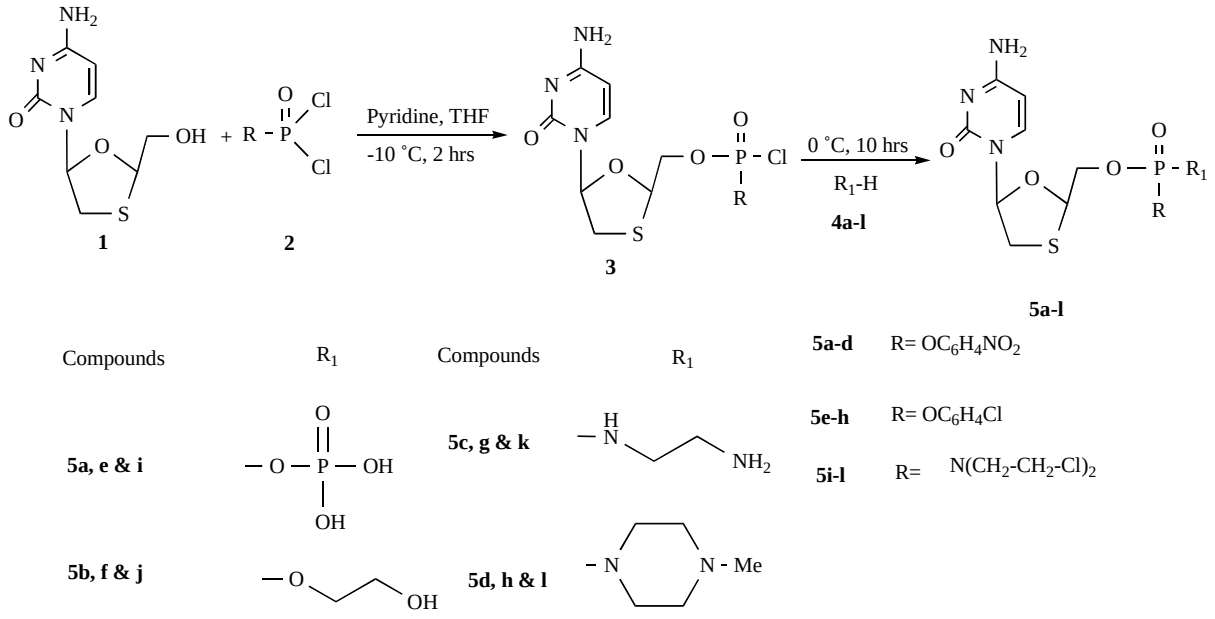
In vitro Experiments

The newly synthesized compounds **5a-5l** in the present study were evaluated for their anti-proliferative activity against COLO-205 cell line by the following procedure. The COLO-205 cells were grown in suspension in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were sub cultured twice per week, seeding at a density of about 2x10^5 cells/mL. For treatment, exponentially growing COLO-205 cells were collected and resuspended in fresh culture medium with 10% FBS. Before the treatment with test compound, cells were washed with PBS and fresh medium was added.

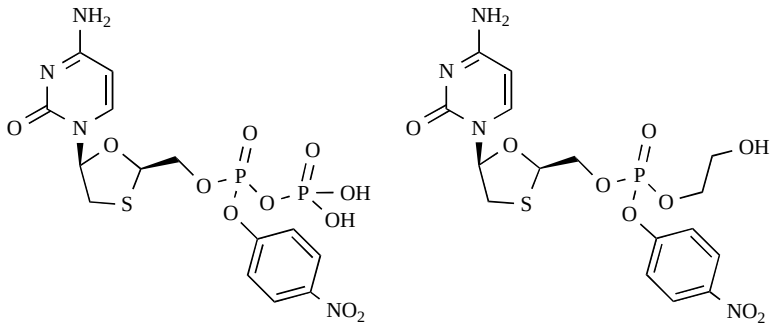
Cell proliferation was assayed by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay as described by Mosmann (1983) [16]. Cells (5×10^3 cells per well) were incubated in 96-well plates in the presence or absence of isolated compound for 24 hrs in a final volume of 100 µL. At the end of the treatment, 20 µL of MTT (5 mg/mL in PBS) was added to each well and incubated for an additional 4 h at 37 °C. The purple-blue formazan precipitate was dissolved in 100 µL of DMSO and the optical density was measured at 570 nm on Quant Bio-tek Instrument, Inc, micro titer plate reader. Data are expressed as mean ±SE. (n=3) % inhibition. Percentage growths in the treated samples were calculated with respect to control. The concentration of the compound that inhibited cell growth by 50% (IC_{50}) was determined from cell survival plots. The results are summarized in Table **1** and anti - proliferative activity of active compounds against COLO- 205 cell line with IC_{50} values are graphically represented in Fig. (**2**), and the values are tabulated in Table **1**.

RESULTS AND DISCUSSION

The synthesis of phosphorylated derivatives of lamivudine (Scheme **1**) was carried out as follows. 4-Amino-1-(2-(hydroxymethyl)-1, 3-oxathiolan-5-yl) pyrimidin-2(1H)-one was treated with 4-nitrophenyl phosphorodichloridate in the presence of triethylamine (TEA) in tetrahydrofuran (THF) and pyridine at -10 ºC. The reaction progress was monitored by thin layer chromatography (TLC) (ethylacetate: hexane 1:1) to form the monochloride **3a**. Further, the monochloride was reacted with ethylenediamine/ ethyleneglycol/ N-methylpiperazine/ mono potassium dihydrogen phosphate to get **5a-d** in high yields. Similarly the same



Scheme 1. Synthesis of lamivudine (3TC) derivatives **5(a-l)**.



5a **5b**

Fig. (1). The structures of the potent lamivudine **(3TC)** phosphorylated derivatives **5a** and **5b**.

above protocol was employed for the synthesis of **5e-l** by using 4-chlorophenyl phosphorodichloridate/ bis (2-chloroethyl) phosphoramidicdichloride. The two corresponding intermediate monochlorides (**3b** and **3c**) were further reacted with mono potassium dihydrogen phosphate, ethylene glycol, ethylenediamine and N-methyl piperazine in the presence of TEA to obtain the target compounds in good yields (69-80%). The purity of compounds was checked by TLC and elemental analyses, and the compounds of this study were identified by spectral data. Characteristic IR stretching absorptions were observed in the regions 1218-1235 cm^{-1}, 3278-3316 cm^{-1} and 3388-3415 cm^{-1} for P=O, N-H [17] and O-H [17] respectively. In ^{1}H NMR spectra of **5a-l,** the chemical shifts of aromatic hydrogen atoms of the phenyl ring were observed as two doublets in the region δ 6.52-7.99 [18]. The N-H proton resonated as a broad multiplet at δ 7.80-7.94 and P-O-H protons were observed as singlet at δ 8.10-9.60. ^{31}P-NMR signals of **5a-l** were observed in the region -7.11 to -19.39 ppm [19]. ^{13}C NMR chemical shifts for compounds **5a-l** were observed in their expected regions [20].

All compounds were screened for their *in vitro* anti colon cancer activity against COLO-205 cell lines by MTT method and IC_{50} values of the synthesized compounds along with the parent drug (Lamivudine) for comparison are reported (Table **1**). The obtained results revealed that the compounds **5a-5c** exhibited good anti colon cancer activity and compounds **5a** and **5b** were identified as the most potent compounds presented in Fig. (**2**) (IC_{50} values 0.003 μM and 0.0001 μM respectively) and exhibited more potent activity than lamivudine against COLO-205 cell line. In this series the most active compounds were **5a, 5b** and **5c** than the reference drug against COLO-205 cell lines. 4-Nitro phenyl phosphato, 2-hydroxyethyloxy and dihydrogen phosphate containing derivatives in Fig. (**1**) (compounds **5a** & **5b** respectively) exhibited promising activities against COLO-205 cell line (Table **1**).

Table 1. ***In Vitro*** **Antiproliferative Activity Values of Lamivudine Derivatives 5(a-e) Against** COLO-205 **Cells**

Compound	IC_{50} (μM)
5a	0.003
5b	0.0001
5c	0.31
5d	32
5e	>100
5f	>100
5g	>100
5h	>100
5i	>100
5j	>100
5k	>100
5l	>100
Reference (Lamivudine) [3TC]	0.425

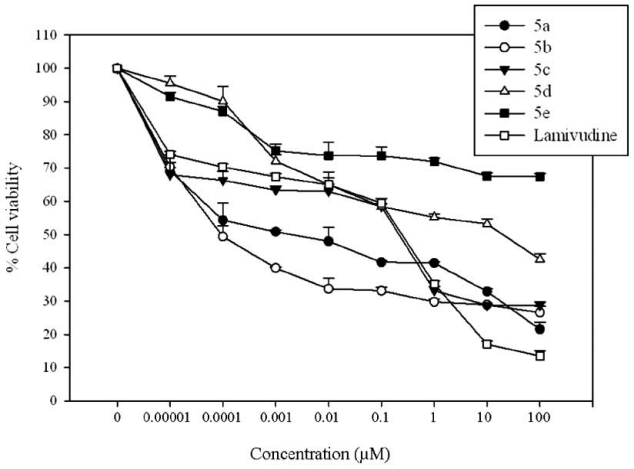


Fig. (2). Growth curves of COLO-205 cells against lamivudine (3TC) phosphorylated derivatives. Reference compound is lamivudine. Data is expressed as mean ±SE. (n=3).

CONCLUSION

In summary, this work describes the preparation and *in vitro* anti colon cancer evaluation of novel phosphorylated derivatives prepared by reaction of lamivudine with phosphorus dichlorides. Three of these compounds (**5a, 5b** and **5c**) were more active than the reference drug against COLO-205 cell line, other derivatives also displayed moderate activities against the COLO-205 cell line. It is conceivable that these derivatives showing anti-colon cancer activity can be further modified to exhibit better potency than the standard drug (3TC). It seems that the anti colon cancer activity de-

pends on the nature of bioactive groups attached to the phosphorus atom, as the best results were obtained with 4-nitro phenyl phosphato, dihydrogen phosphato (**5a**) and 2-hydroxyethyloxy (**5b**) moieties.

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