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Synthesis of novel phosphorylated derivatives of Tenefovir Intermediate and their antiviral activity

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ABSTRACT

A series of new phosphorylated compounds **5a-j** were synthesized by the sequence of chemical reactions. Initially, Tenofovir intermediate **(1)** was treated with 4-chlorophenyl phosphorodichloridate **(2)** to form an intermediate **3.** Secondly, the intermediate **3** on reaction with various amino acid esters (**4)** in presence of dry THF/Py and *N,N*-dimethyl piperazine afforded the title compounds**.**The spectral data and elemental analyses were confirmed to the title compounds **5a-j** and further tested for their antiviral activity against with reference standards Blue Tongue Virus (BTV) and New Castle disease Virus (NDV). The **5e, 5g** and **5i** compounds have exhibited potent activity against BTV and **5b, 5d, 5e, 5f** and **5h** have shown moderate activity against NDV.

1. Introduction

The ANPs are nucleotide analogs and form stable P-O bond through side aliphatic chain with phosphorus [1]. These exhibit cytostatic [2], anti-parasitic [3] and stimulates the immune system properties [4]. Mainly, cidofovir, adefovir and tenofovir drugs are active portion of effective antiviral agents used for treatment of viruses caused diseases are hepatitis B and AIDS [5]. These nucleosides abilities are dependent on their biolgical evaluation of cellular kinases to the respecting mono, di and triphosphates [6]. The aminoacid ester bonded of P-O at phosphorous compounds are essential category of irrationally designed and synthesized medicine possess anti-neoplastic activities [7]. The researchers are focused on to design and development of therapeutic drugs by using the phosphorylation step. This method has better results in the synthesis of new potent drugs of nucleotide derivatives [8, 9]. Some of the prodrugs are proven for the treatment of anti viral infections [10]. Mono-phosphoramidate or bis-amidate nucleosides of prodrugs exhibited anti HIV properties [11]. Cyclic nucleoside phosphonate is found to inhibit HIV reverse transcriptase and their derivative of the prodrug as a auspicious drug [12]. Ballatore *et al.* reported the phosphonamidate prodrugs of tenofovir found to better results than ANPs [13], and prodrug of tenofovir enhances the antiviral properties [14]. Isopolar phosphonomethyl group having nucleotide derivatives have enzymatic problems controlled by intra cellular phophorylation of nucleoside stimulation. Particularly, the compounds [(R)-2-phosphonomethoxypropyl]adenine's are active in opposition to retroviruses [15]. The modified nucleotide's are exhibiting virus-inhibitory activity, which subsequently act as terminators of the growing DNA chain [16]. In this view, we have great interest to report a class of new phosphorylated

derivative of tenofovir intermediate and examine their antiviral properties connected with reference standards.

2. Experimental method

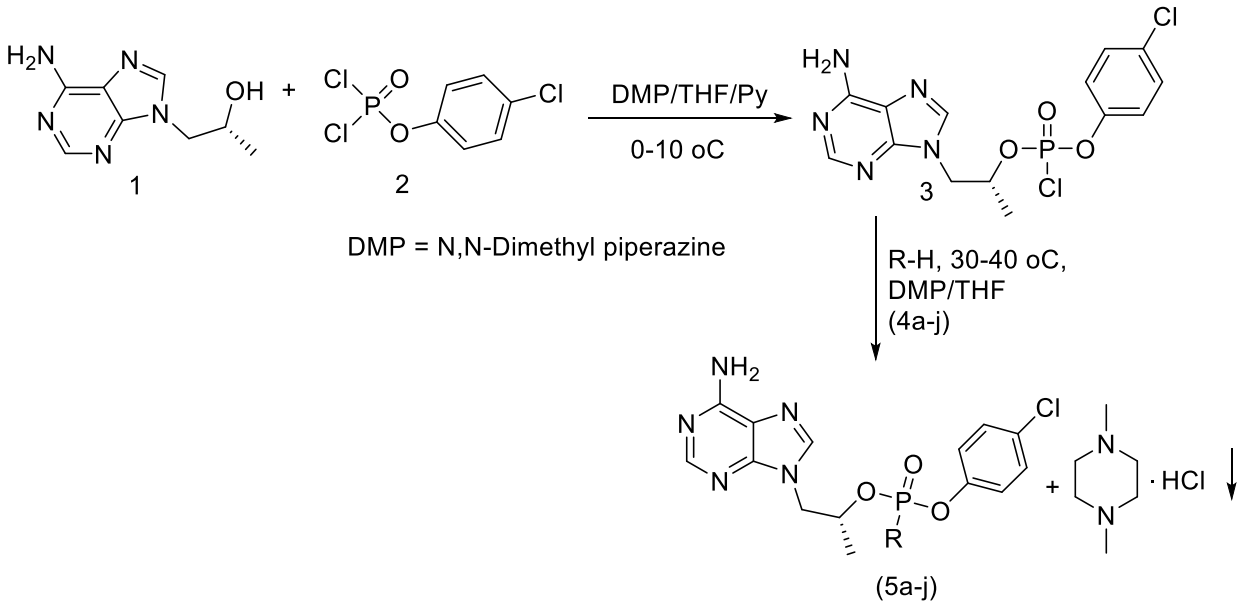
2.1. Chemistry

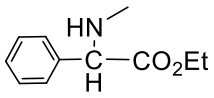
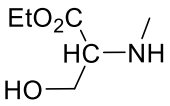
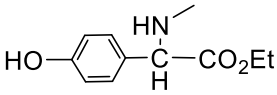
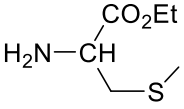
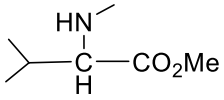
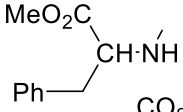
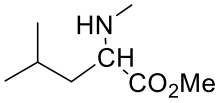
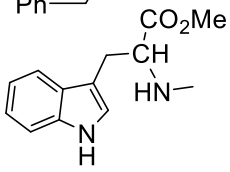
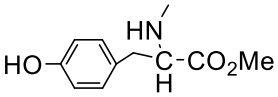
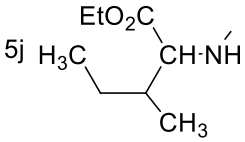
The purchased chemicals were taken as of sigma Aldrich and Merck company for without further purification. According to literature methods [17], all the solvent should maintain the spectroscopic and reagent grade before carryout the reactions.

The instruments of Perkin-Elmer 283 unit were recorded of IR spectra in the presence of KBr pellets. The (^{1}H, ^{13}C) NMR spectra's were record at 400 MHz and 100 MHz for operating frequencies and a solvent DMSO-d_6 referenced to TMS, 161.9 MHz for ^{31}P NMR (85% H_3PO_4). The molecular ion peaks were recorded spectrometer on a LC-MS of Jeol SX 102 DA/600. The Instrument of Thermo Finnigan recorded of elemental analysis at University of Hyderabad.

Synthetic procedure for the title compounds **5(a-j).**

The compound **1** (0.001 mol) liquefy in an appropriate amount of Tetrahydrofuran/Pyridine (20 mL) in the ratio of 2:1 were taken in a RB, stirred & cooled at 0-10 ºC. To this stirred solution, 1, 4-dimethyl piperazine (0.001 mol), para-chlorophenyl phosphorodichloridate **(2)** (0.001 mol) was added slowly, enhanced the temperature to room temperature and stirred for 1h resulted the formation of an intermediate **3** and *N,N*- dimethyl piperazine. HCl salt. The salt (*N,N*- dimethyl piperazine hydrochloride) was detached through the filtration and the steps forward of the result was monitor by Thin layer chromatography using $CHCl_3$: MeOH (3:1) as an eluent. Further, the intermediate **3** was treated with phenyl glycine ethyl ester (**4)** in the presence of base (*1, 4*- dimethyl piperazine) in dry THF at 30−40 ºC and stirred for 2−4 h resulted the formation of crude mixture containing *N,N*- dimethyl piperazine.HCl. The above uncompleted salt was impassive and the solvent was vanishing by rota-evapoartor. The crude compound was purified by column using 5:1 ratio of $CHCl_3$: MeOH as an eluent afforded a pure compound **5a**. The same methodology applies to the synthesis of left over select compounds **5b-5j** represented in the Scheme 1.



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

3. Results and Discussion

3.1. Synthesis

A series of new phosphorylated esters **5(a-j)** was accomplished by the reaction of Tenofovir intermediate (**1)** with para-chlorophenyl phosphorodichloridate in the presence of *1,4*-dimethyl piperazine as a base in THF:py (2:1, solvent) to form an intermediate **3** **®**-1-(6-amino-9*H*-purin-9-yl) propan-2-yl-4-chlorophenylphosphorochloridate**.** The intermediate **3** was further reacted with various aminoacid esters **4(a-j)** in the presence of base with stirring at 30−40 ºC to afford the title compounds **5(a-j)** according to the procedure outlined in **Scheme 1**. The design of the **5(a-j)** compounds was ascertained by TLC using $CHCl_3$: MeOH (3:1). The title compounds **5(a-j)** were purified by column chromatography to obtain 72-84 % yields and in short reaction times 3−5 h. The IR spectral data for the title compounds containing functional groups $-NH_2$, P=O and $P-O-C_{aliph}$ stretching vibrations in the regions of 3446−3412, 1234−1220 and 1032−1012 cm^{-1} respectively. In the ^{1}H-NMR spectra, a broad singlet at δ 10.50−10.12 is assigned to adenine -NH_2. The aminoacid ester P−NH is resonated as a triplet in the range of 4.40−4.56 ppm (*t*, *J* = 5.2−6.4 Hz). The aromatic protons resonated in the region 7.48−6.52 ppm.^{31}P-NMR spectra of chemical shifts were observed in the region of 21.4−25.6 ppm [21].

3.2. Antiviral activity

The synthesize compounds exhibited antiviral activity against reference standards NDV and BTV *in vivo* and *in vitro* studies. Effect of Blue tongue virus (BTV) symptoms on the embryo were observed +++ for best active, ++ for better active and + for low active. The tested title compound exhibit activities against BTV and New castle disease virus (NDV) are shown in **Table 1** and **Table 2**. Based on above biological assay, **5g** and **5i** exhibited excellent activity compared to remaining title compounds and equal to standard reference compound. It may be due to the presence of cysteine ethyl ester and tryptophan methyl ester substituents at phosphorus atom. The compounds **5b, 5d, 5e, 5f** and **5h** exhibited moderate antiviral activity compared to standard reference compound. In this biological assay, $TCID_{50}$ values were calculated by using BHK-21 cells from BTV stocks and Haemagglutination (HA) test.

Table 1: The activity of **5(a-j)** compounds with against NDV.

Compounds	No of eggs	Mortality				HA Test	
		24 h	48 h	72 h	96 h	+ve	-ve
5a	5	0/5	0/5	1/5	0/4	1	4
5b	5	0/5	1/5	2/5	0/2	2	3
5c	5	0/5	1/5	1/5	0/2	2	3
5d	5	0/5	0/5	1/5	0/4	1	4
5e	5	0/5	1/5	1/5	0/3	2	3
5f	5	0/5	0/5	1/5	0/4	1	4

5g	5	0/5	1/5	1/5	1/5	3	2
5h	5	0/5	0/5	1/5	0/4	1	4
5i	5	0/5	1/5	1/5	1/5	3	2
5j	5	0/5	0/5	1/4	0/4	1	4
[a]Positive control	5	0/5	0/5	0/5	0/5	0	5

[a]Positive control: tenefovir intermediate

Table 2: Antiviral activity report of 5(a-j) with BTV

Compounds	BTV symptoms in (embryonated eggs)	BTV infected BHK 21 Cells (% of live cells)
5a	+	62
5b	++	79
5c	+	65
5d	++	75
5e	+++	82
5f	++	77

5g	+++	83
5h	++	72
5i	+++	86
5j	+	67
Positive control[a]	**+++**	91

Positive control[a]: tenofovir intermediate.

4. Conclusions

We have synthesized a series of new **5(a-j)** compounds have in good yields and short reaction period. The synthesized compounds exhibit moderate antiviral activity, when compared to BTV and NDV *in vitro* and *in vivo* assay.

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