Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Original article

Synthesis, structure–activity relationship and antiviral activity of 3’-N, N-dimethylamino-2’,3’-dideoxythymidine and its prodrugs

Ramendra K. Singh a,*, Dipti Yadav a, Diwakar Rai a, Garima Kumari a, C. Pannecouque b, Erik De Clercq b

a Nucleic Acids Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad 211002, India
b Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

A R T I C L E   I N F O

Article history:
Received 4 March 2010
Received in revised form 11 May 2010
Accepted 12 May 2010
Available online 20 May 2010

Keywords:
Dideoxythymidine
NRTIs
VSV
HIV-1
HIV-1 RT
MTT assay

A B S T R A C T

A probable NRTI molecule, viz. 3’-N,N-dimethylamino-2’,3’-dideoxythymidine (4) and its 5’-O-carboxyl ester prodrugs – 5’-(N,N-BOC-O-phenylalanyl)-3’-N,N-dimethylamino-2’,3’-dideoxythymidine (5), 5’-(N,N-BOC-O-phenylalanyl-3’-N,N-dimethylamino-2’,3’-dideoxythymidine (6) and 5’-decanoyl-3’-N,N-dimethylamino-2’,3’-dideoxythymidine (7) have been synthesized and screened against HIV, HSV-1 and 2, parainfluenza-3, vesicular stomatitis and several other viruses. The compound 6 showed good antiviral activity with EC50 value 0.03 μM (SI = 8) against VSV in Hela and HEL cell lines. However, the lead compound 4 and its derivatives 5, 6 and 7 showed no remarkable activity against HIV-1 and other viruses. Molecular docking studies with HIV-1 RT using DS 2.5 and pymol softwares have shown marked differences in the interaction patterns between the lead compound 4 and AZT.

© 2010 Elsevier Masson SAS. All rights reserved.

1. Introduction

HIV-1 reverse transcriptase (HIV-1 RT), a primary target for developing anti-HIV drugs, is the enzyme responsible for generating linear dsDNA from ssRNA – the genetic material of HIV-1. This dsDNA acts as a provirus and leads to viral expression through integration in the host genome, transcription and translation processes.

There are several drugs that inhibit HIV-1 RT and they are classified as nucleos(t)ide RT inhibitors (N(t)RTIs) and non-nucleoside RT inhibitors (NNRTIs). The NRTIs have emerged as important processes.

The NRTIs, by virtue of acting as chain terminators of (−) strand DNA synthesis during reverse transcription, have been developed as potential drugs against HIV/AIDS. In order to explore such new inhibitors, we report here the synthesis of 3’-N,N-dimethylamino-2’,3’-dideoxythymidine (DMAT) and its 5’-O-ester prodrugs with amino and fatty acids. The lead molecule, DMAT, was designed keeping AZT, an approved NRTI drug against HIV/AIDS, in mind, where azido group has been replaced by dimethylamino function, Fig. 1. Thus, this molecule being a dideoxynucleoside is expected to act as NRTI against HIV-1 RT enzyme. Similarly, the prodrugs, having biodegradable ester linkages, are supposed to enhance the cellular uptake due to their high lipophilic nature.

All these molecules have been evaluated for their activity against various viruses, like HIV-1, vesicular stomatitis, HSV-1 & 2, reovirus, parainfluenza virus and many others.

10.1016/j.ejmech.2010.05.028
2. Chemistry

A new thymidine analogue, viz. 3’-NN-dimethylamino-2’,3’-dideoxythymidine has been synthesized from thymidine as shown in Scheme 1. 5’-O-(4,4’-Dimethoxytrityl)-3’-mesitylmethythymidine, obtained after 5’-OH selective protection with DMTCI followed by mesylation with MsCl, when reacted with potassium phthalimide witnessed the introduction of nucleophile (phthalimide ion) into the sugar moiety [18]. During conversion of 1→2, the elimination of 3’-mesolox group was effected through intramolecular displacement by attack of carbonyl group of thymidine at C2, which resulted in formation of 2,3’-anhydro-nucleoside intermediate. This result was well in accordance with the literature data. It is suggested that under this condition, phthalimide ion is introduced into the ‘down’ (ribo) configuration [19]. The 3’-phthalimide derivative 2 was treated with methanolic methylamine, which through methanolyis and aminolysis resulted in formation of compound 3 [20]. Reaction of 3 with formic acid and aqueous formaldehyde (40%) yielded 4. In this reaction, formaldehyde used was the source of methyl groups, while formic acid supplied the hydrogens involved in the reduction. This treatment also removed the DMT group from compound 3. As expected, the UV absorption spectra of all intermediates were similar to that of thymidine, while dissimilar R1 values showed that all these changes took place in the sugar moiety and not in the aglycon part [19].

3. Results and discussion

3.1. Antiviral activity

All compounds 4–7 have been screened for their antiviral potential against HIV and several other viruses and the results are shown in Table 1. The lead compound DMAT and its prodrugs were found inactive against HIV-1 ROD and HIV-1 IIIB strains and showed toxicity. Compounds 4 and 5 have shown similar values of CC50 and EC50 against all viruses studied and thus no activity was observed, while 5’-a-phenylalanyl derivative 6, having free amino function was potentially active at an EC50 of 0.03 μM against VSV in HeLa and HEL cell cultures. The EC50 value of compound 6 has been found to be eightfold lower than its CC50 value (SI = 8). Besides this, it has also shown moderate activity at an EC50 of around 0.05 μM against HSV-1 & 2, coxackie B4, and RSV in HeLa cell culture. At the same EC50 value, it was found active against VV, TK HSV, in HEL cell lines; PIV-3, RV-1, SV and PTV in Vero cell culture and ECV (FIPV) and FHV in CRFK cell culture. The 5’-decanoyl derivative 7 has shown some activity against FCV (FIPV) and FHV in CRFK cell culture.

All compounds were also tested against influenza A H1N1/H3N2 subtype and Influenza B in MDCK cell lines. However, none of these compounds was able to inhibit cytopathic effects of Influenza A or B virus at subtoxic concentrations or the highest concentration tested.

It was observed that compounds 6 and 7 only, showed antiviral properties against VSV (EC50 0.03) and FCV (EC50 0.05), respectively. However, the antiviral potency of the produg 6 was still significantly lower than that of established antiviral drugs — (S)-DHPA, BDVU and ribavirine against VSV as was evident from its CC50 and EC50 values shown in Table 1.

Since nucleosides exhibit only modest bioavailability [21], we prepared the produg molecules using amino and fatty acids to improve their bioavailability [22,23] and chemical stability. This was done to ensure enhanced cellular uptake and sustained release of drug molecule without compromising its properties [24–27].

The lead compound 4 and its produg 5 did not show any antiviral activity, whereas the produg 7 showed activity, albeit, too little against FCV and FHV. However, the produg 6 showed good activity against VSV with SI value equal to 8. The enhanced reactivity of free amino acid ester produg may be attributed to its electronic activation by the positively charged amino terminus at physiological pH, which derives support from the fact that l-amino acid esters enhance nucleoside absorption via human peptide transporters (hPEPT1) of active transport system [28,29]. Furthermore, since bone marrow progenitor cells lack this active transport system for amino acid, produg 6 is expected to show reduced toxicity at bone marrow level. Therefore, compound 6 has potential to be developed as an effective antiviral agent against VSV.

The lead compound 4, being a 2’,3’-dideoxynucleoside, was expected to act as chain terminator and thus, interfere with viral cell metabolism and its prodrugs were designed and synthesized to enhance its cellular uptake. However, this molecule showed almost no anti-HIV activity and rather proved cytotoxic. This might be
Table 1
Antiviral and cytotoxic effects of compounds 4–7.

| Compound   | Virus                  | Cell | HeLa | HeLa | HEL | HEL | HEL | HEL | HEL | Vero | Vero | Vero | Vero | CRFK | CRFK | CRFK | MDCK | MDCK | MDCK | MTT | MTT |
|------------|------------------------|------|------|------|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|
| 4          | EC$_{50}$              | 0.03 | 0.20 | 0.20 | 0.20 | 0.05 | 0.05 | 0.30 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |
| 5          | EC$_{50}$              | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| 6          | EC$_{50}$              | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| 7          | EC$_{50}$              | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| (S)-DHPA   | EC$_{50}$              | 146  | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| Ribavirin  | EC$_{50}$              | 10   | 30   | 30   | 10   | 50   | 50   | 85   | 125  | 95   | 250  | 250  | 250  | 250  | 250  | 250  | 250  | 250  | 250  | 250  | 250  | 250  |
| Brivudine  | EC$_{50}$              | 0.08 | 50   | 2    | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 | >250 |
| Ganciclovir| EC$_{50}$              | 0.03 | 0.03 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| Oseltamivir| EC$_{50}$              | 0.07 | 1.7  | 4    | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   | 45   |
| Amantidin  | EC$_{50}$              | 20   | 0.8  | 100  | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| Rimantidin | EC$_{50}$              | 100  | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |

a – Vesicular stomatitis virus; b – coxsackie virus B4; c – respiratory syncytial virus; d – herpes simplex virus-1(KOS); e – herpes simplex virus-2 (G); f – vaccinia virus; g – vesicular stomatitis virus; h – herpes simplex virus-1 TX-KOS ACV; i – para-influenza-3 virus; j – reovirus-1; k – sindbis virus; l – coxsackie virus B4; m – punta toro virus; n – feline corona virus (FPV); o – feline herpes virus; p – influenza A H1N1 subtype; q – influenza A H3N2 subtype; r – influenza B; s – HIV-1 ROD; t – HIV IIIb.

EC$_{50}$ – compound concentration (in µM) required to reduce virus yield by 50%, CC$_{50}$ – compound concentration (in µM) required to reduce cell viability by 50%.
expected because of introduction of dimethylamino group at 3′-C of ribose moiety, which did not show any interaction with HIV RT during molecular docking experiments. The lead molecule 4 was designed because of its similarity with AZT, but it completely failed in fulfilling the objectives. It was surprising to see that replacement of azido group by dimethylamino group turned the molecule highly toxic.

### 3.2. Molecular docking

The fact that the lead molecule DMAT (and its prodrugs) being a dideoxynucleoside and having structural features similar to AZT, did not show any activity against HIV and rather proved toxic, prompted us to study its interaction with HIV-1 RT.1 We performed elaborate molecular computational studies using the software Discovery Studio (DS) 2.5. This molecule showed some striking differences with AZT − the approved anti-HIV drug as shown in Table 2. The physiochemical studies suggested that both the molecules − AZT and DMAT, followed Lipinski’s rule of five but they have marked differences in their molecular volume, total polar surface area (TPSA) and log P values. Similarly, the docking experiment also showed quite different patterns of their interaction with HIV-1 RT (Figs. 2−4). Although DMAT formed one more H-bond with HIV-1 RT than AZT, the crucial interactions as shown by AZT were missing in the case of DMAT. The azido function on AZT formed three H-bonds − one with Arg72 and two with Asp113 (the amino acid constituting the dNTP-binding site), whereas the dimethylamino function on DMAT formed no H-bonds at all. Similarly, the H-bond formed by phosphate moiety of AZT (and TTP as well) with Val111 was missing in the case of DMAT. However, the aglycan part in both the molecules formed two H-bonds with Arg72 through oxo group and “O” atom in the sugar ring. The stability of DMATTP−RT complex was also less than that of AZTTP−RT or TTP−RT complexes as is evident from their minimization energies. A comparative account of interaction of TTP, AZTTP and DMATTP and stability of their respective complexes with HIV-1 RT has been shown in Table 3. Thus, the physiochemical and docking studies suggested the possible reasons for inactivity of the lead molecule and its prodrugs.

### 4. Conclusion

In conclusion, we have synthesized 3′-N,N-dimethylamino-2′,3′-dideoxythymidine 4 and its 5′-O-carboxyl ester prodrug derivatives 5−7 and evaluated their antiviral activity against HIV-1 (IIIG and ROD strains), HSV-1/2, vesicular stomatitis, parainfluenza-3 virus and many others. It was observed that compounds 6 and 7 only, showed moderate antiviral properties against VSV (EC50 0.03) and FCV (EC50 0.05), respectively. Further mechanistic studies on molecules 6 and 7 are in progress against vesicular stomatitis, feline corona and feline herpes viruses.

The inference drawn from the present studies that for a nucleoside analogue to be an effective NRTI, being a dideoxy derivative is not sufficient enough criterion and rather it must possess the characteristic features too for requisite interactions at the dNTP-binding site of HIV-1 RT enzyme shall definitely help in designing new NRTI molecules.

### 5. Experimental

#### 5.1. Chemistry

Chemicals were obtained from E. Merck India Ltd, India and Sigma−Aldrich Chemical Company, USA. Melting points were determined on electrotherm apparatus and are uncorrected.

### Table 2

| Molecules | M.W. (g/mol) | Mol. vol. (Å3) | No. of H-acceptors | No. of H-donors | TPSA (Å2) | log P |
|-----------|--------------|----------------|--------------------|----------------|-----------|-------|
| Thymidine | 242.231      | 207.189        | 7                  | 3              | 104.557   | −1.433|
| AZT       | 267.245      | 224.063        | 9                  | 2              | 134.084   | −0.099|
| DMAT      | 269.301      | 245.078        | 7                  | 2              | 87.567    | −0.771|

Minimization energy: (−) 63.93805 kcal/mol, Number of H-bonds formed-13
(Phosphate (9) – Val 110, Val 111; C=O of base (2) – Arg 72; 3′ OH (1) – Asp 113)

Fig. 2. Interaction of TTP with HIV-1 RT at dNTP-binding site.

Fig. 3. Interaction of AZTTP with HIV-1 RT at dNTP-binding site.

1 This facility was not available in the beginning.
Minimization energy: (-) \(61.20318\) kcal/mol, Number of H-bonds=14

Fig. 4. Interaction of DMATTP with HIV-1 RT at dNTP-binding site.

Silica gel for TLC and column chromatography (60–120 mesh) was obtained from E. Merck India Ltd. UV measurements were carried out on Hitachi 220S spectrophotometer. HPLC analyses were performed using 250 × 4.6 mm² C18 column and solvent system MeOH:H₂O (6:4) at a flow rate of 1 ml/min on 6 AD Binary Gradient Shimadzu HPLC system. ¹H NMR spectra were recorded in DMSO-\(d_6\) on a Varian XL-300 spectrometer operating at 75 MHz. Mass spectra were obtained using a Thermofinnigan TRACE-DSQ Electrospray Ionization (ESI) mass spectrometer. Elemental analyses were carried out on a Perkin–Elmer 240-C analyzer. All solvents were dried and distilled prior to use.

5.1.1. 5’-O-(4,4’-Dimethoxytrityl)-3’-phthalimidothymidine (2)

Thymidine (2.415 g, 10 mmol) was suspended in dry pyridine (≈ 100 ml) in a 250 mL round bottomed flask and added DMAP (61 mg, 0.05 equivalent), TEA (1.9 ml, 1.4 equivalent) and DMTCI (4.1 g, 1.2 equivalent) and stirred the reaction mixture for 2.5 h. TLC at this point showed complete disappearance of thymidine. The contents were cooled to 0°C and methanesulfonyl chloride (1.23 ml, 10.89 mmol) was added dropwise. Stirring was continued further for 2 h and the reaction mixture was allowed to warm up to 25°C. After stirring the reaction mixture for an additional 30 min, the contents were poured into crushed ice/water and the viscous mass so obtained was extracted in chloroform (50 ml) and washed with water (2 × 10 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated on a rotary evaporator to obtain the crude product. Silica gel column purification with a linear gradient up to 10% ethyl acetate in hexane, afforded the pure compound, 5’-O-(4,4’-dimethoxytrityl)-3’-mesyl-2’,3’-dideoxythymidine, which was refluxed for 12 h with potassium phthalimide (9.0 g) in dimethylformamide (450 ml). The solvent was removed under reduced pressure and the residue extracted with ethyl acetate. The ethyl acetate layer was finally washed with water (3 × 10 ml), dried over anhydrous Na₂SO₄ and concentrated to get the title compound in crude form (4.72 g, 70%).

5.1.2. 5’-O-(4,4’-Dimethoxytrityl)-3’-amino-2’,3’-dideoxythymidine (3)

The compound 2 (4.34 g) was stirred with methylvamine (16 ml) in methanol at 105°C for 20 h. The reaction mixture was evaporated to an oily residue, washed with water, dissolved in ethanol (100 ml) and treated with hydrochloric acid to neutralize the residual methylvamine. The acidic solution was concentrated under reduced pressure, which resulted in crystallization. The crystals were separated and the residual water and acids were removed by co-evaporation with benzene under reduced pressure from the mother liquor, which was further subjected to crystallization. The crystalline residue was triturated with ethanol and ether. The title compound was obtained as colorless crystals (3.04 g, 87%).

5.1.3. 3’-NN-Dimethylamino-2’,3’-dideoxythymidine (4)

5’-O-(4,4’-Dimethoxytrityl)-3’-amino-2’,3’-dideoxythymidine (3.04 g, 5 mmol) dissolved in a mixture of 98—100% formic acid (6 ml) and 40% aqueous formaldehyde (6 ml) was refluxed at 70°C for 20 min to give light orangish syrup. The reaction mixture was concentrated to obtain the crude product. Silica gel column purification with a linear gradient up to 10% ethyl acetate in hexane, afforded the pure compound, 5’-O-(4,4’-dimethoxytrityl)-3’-amino-2’,3’-dideoxythymidine (3.04 g, 5 mmol) dissolved in a mixture of 98—100% formic acid (6 ml) and 40% aqueous formaldehyde (6 ml) was refluxed at 70°C for 20 min to give light orangish syrup. The reaction mixture was concentrated to obtain the crude product.

Table 3

Interaction of Thymidine, AZT and DMAT triphosphates with HIV-1 RT.

| Molecule   | Total number of H-bonds | Bonds formed with PO₄ unit | Bonds formed with C=O on base | Bonds formed with sugar moiety | Bonds formed with 3’ functional group | Minimization energy (kcal/mol) |
|------------|-------------------------|----------------------------|-----------------------------|-------------------------------|----------------------------------------|-------------------------------|
| TTP        | 11                      | 8 Val110, 111              | 1 Arg72                     | 1 Arg72                       | 1 (OH) Asp113                         | -61.20318                     |
| AZTTP      | 13                      | 8 Val111, 10 Asp113,      | 1 Arg72                     | 1 Arg72                       | 3 (Nâ) Arg72, Asp113                  | -63.93809                    |
| DMATTP     | 14                      | 10 Asp113, Glu44, Lys46   | 1 Arg72                     | 0 (N−(CH₂)₂)                  | -53.81129                             | -53.81129                    |
5.1.5. \(5'-\text{a-Phenylalanyl-3',N'-dimethylamino-2',3'-\text{dideoxythymidine}}\) (6)

Compound 5 (150 mg, 0.30 mmol) was added slowly to a mixture of 30% TFA and methylene chloride (10 ml). The solution was stirred under anhydrous condition for 30 min. The solvent was removed under vacuum and the residue treated with triethylamine in methylene chloride to afford the title molecule. The product was purified by silica gel column chromatography using ethyl acetate/methanol (4:1) as eluent. Yield: (80 mg, 63%) mp 219–220 °C; \(t_{R}: 0.29\) (DCM:MeOH 9.5:0.5) UV (EtOAc) \(\lambda_{\text{max}}\) 265 nm. \(^{1}\)H NMR (DMSO-d6): \(\delta 1.94\) (s, 3H, 5-CH3); 2.15 (m, 2H, H-2); 2.26 (s, 6H, \(-\text{N} (\text{CH}_3)_2\)); 2.95 (m,1H, H-3'); 3.18 (d, 2H, CH2-amino acid); 3.86 (t, 1H, CH-amino acid); 4.20 (m, 2H, H-5'); 4.58 (m,1H, H-4'); 5.85 (m, 1H, H-1'); 7.26–7.08 (m, 5H, Ar); 7.55 (s, 1H, H-6); 10.05 (s, 1H, NH, Thy); \(^{13}\)C NMR (CDCl3): \(\delta 15.8, 31.5, 33.3, 39.2, 57.9, 59.5, 60.6, 67.2, 74.6, 78.5, 109, 127.8, 128.1, 125.4, 134.8, 140.9, 127.7, 152.2, 172;\) ESI-MS m/z (M+•) 488. Anal. calcd for C25H36N4O6: C, 61.46; H, 7.43; N, 11.73; found: C, 61.15; H, 7.30; N, 11.47.

5.1.6. \(5'-\text{Decanoyl-3',N'-dimethylamino-2',3'-\text{dideoxythymidine}}\) (7)

Decanoyl chloride (0.20 ml, 1 mmol) was added dropwise to an ice-cold stirred solution consisting of the lead compound 4 (269 mg, 1 mmol), DMAP (0.32 g, 2.6 mmol) in pyridine (25 ml) and the reaction mixture stirred overnight at room temperature under anhydrous condition. Reaction mixture was treated under reduced pressure and partitioned between ethyl acetate and water. The organic fraction was concentrated to a residue and the product was purified by column chromatography over silica gel using ethyl acetate/hexane (9.5:0.5) as eluent. Yield: (200 mg, 59%) mp 160 °C; \(t_{R}: 0.84\) (DCM:MeOH 9.5:0.5); UV (EtOAc) \(\lambda_{\text{max}}\) 265 nm, C18 HPLC (265 nm) \(t_{R}: 2.84\) min; \(^{1}\)H NMR (DMSO-d6) \(\delta 0.97\) (t, 3H); 1.29 (m, 10H); 1.33 (q, 2H); 1.68 (m, 2H); 1.93 (s, 3H, 5-CH3); 2.13 (m, 2H, H-2'); 2.25 (t, 2H); 2.27 (s, 6H, \(-\text{N} (\text{CH}_3)_2\)); 2.97(m,1H, H-3'); 4.21 (m, 2H, H-5'); 4.57 (m,1H, H-4'); 5.85 (m, 1H, H-1'); 7.22–7.18 (m, 5H, Ar); 7.54 (s, 1H, H-6); 10.03 (s, 1H, NH, Thy); \(^{13}\)C NMR (CDCl3): \(\delta 15.2, 31.5, 39.4, 39.7, 55.2, 60.1, 67.2, 74.1, 78.9, 109.2, 125.7, 128.7, 134.1, 140.9, 152.3, 172.8,\) ESI-MS m/z (M+•) 416. Anal. calcd for C24H36N4O8: C, 61.14; H, 6.91; N, 11.73; found: C, 60.14; H, 4.70; N, 11.61.

5.2. Biological evaluation

5.2.1. Antiviral assays

Antiviral assays were based on inhibition of virus-induced cytopathogenicity in various cell cultures following the established procedures [29–32]. The assays were performed against several viruses, viz. HIV, herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G), cytomegalovirus (CMV), sindbis virus (SV), parainfluenza virus type-3 (PIV-3) and resorvirus type-3, vesicular stomatitis virus (VSV), coxsackie virus (Coxs V) and respiratory syncytial virus (RSV) using CRFK, HEL, HeLa, Vero and CD-3 cell lines.

5.2.2. Virus cytopathogenicity

Cell cultures were prepared in microtiter trays and inoculated with 100 CCID50 (1 CCID50 corresponding to the virus stock dilution that proved infective for 50% of cell cultures). After 1 h virus adsorption to the cells at 37 °C, the residual virus was replaced by cell culture medium (Eagle minimum essential medium supplemented with 3% fetal calf serum) and various concentrations of the test compounds. Virus cytopathogenicity was recorded as it reached completion in the untreated virus-infected cell cultures, i.e., at 1–2 days for vesicular stomatitis virus; 2 days for coxsackie; 2–3 days for vaccinia, herpes simplex type 1 and 2 and sindbis; 4 days for respiratory syncytial virus and 6–7 days for reo and parainfluenza viruses [33,34]. The antiviral activity of compounds is expressed as EC50 (the concentration (µM) required to inhibit virus-induced cytopathogenicity by 50%).

5.2.3. Cytotoxicity assays

Cytotoxicity of all compounds was assessed on the basis of two parameters: (i) alteration of normal cell morphology, and (ii) inhibition of macromolecule (DNA, RNA and protein) synthesis. Cytotoxicity (CC50) of compounds was examined by trypan blue exclusion test. To evaluate cytotoxicity, uninfected confluent cell cultures treated with various concentrations of test compounds were incubated in parallel with virus-infected cell cultures prepared in plastic trays containing 24 wells (16 mm diameter; Falcon plastics). After 2 days of incubation at 37 °C in a CO2 incubator, when the cell cultures were confluent, culture medium was removed from each well and 1 ml of maintenance medium containing serial concentrations of the test compounds was added. For cell control, 1 ml of maintenance medium without compound was added. All cultures were incubated at 37 °C, and after 2 and 7 days of incubation, compounds were withdrawn and the viability of the cells was determined by the trypan blue exclusion method.

5.2.4. Anti-HIV assay

Antiviral screening against HIV-1 (IIIB and ROD strains) was monitored by the efficiency of test compounds to inhibit syncytia formation after HIV infection of MT-4 cells following the MTT method [34–36]. The activity of compounds against HIV-1 was monitored by inhibition of HIV-1-induced cytopathogenicity in MT-4 cells. Briefly, MT-4 cells (3 × 104 cells per well in 96 well plate) were cultured in microtiter trays in presence of various concentrations of test compounds added immediately after infection with 50% cell culture infective doses of HIV-1. After 5 days of incubation at 37 °C, the number of viable cells was determined by the MTT (3-[4,5-dime-thylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) method.

5.2.5. Molecular modeling study

Molecular docking of compound 4 and its prodrugs 5 and 7 into the dNTP-binding site of HIV-1 RT was carried out using DS 2.5 software and PDB code 3E01 of HIV-1 RT. CDocker was used and flexible docking was done. Stimulated annealing was done to get the energy-minimized structures of the molecules. The energy-minimized forms were then docked into HIV-1 RT. Visualization was done using PyMol software. All molecular modeling studies were performed on an Intel Pentium 2.99 GHz processor, 1.99 GB RAM with Windows XP professional version 2002 operating system.

Acknowledgements

Financial assistance from the Department of Biotechnology (DBT) and Indian Council of Medical Research (ICMR), New Delhi, Government of India for carrying out this work is sincerely acknowledged.

References

[1] H. Ali, N. Ahmed, G. Tessier, J.E.V. Lier, Bioorg. Med. Chem. Lett. 15 (2006) 317–319.
[2] D. Genini, S. Adachi, Q. Chao, D.W. Rose, C.J. Carrera, H.B. Kotram, D. Carlson, L. M. Lonig, Blood 96 (2000) 3537–3543.
[3] K.S. Godumundsson, G.A. Freeman, J.C. Drach, L.B. Townsend, J. Med. Chem. 43 (2000) 2473–2478.
[4] Y.S. Lee, B.H. Kim, Bioorg. Med. Chem. Lett. 20 (2002) 1395–1397.
[5] C.M. Calmarini, J.R. Mackey, C. Dumontet, Nature 15 (2001) 875–890.
[6] E.J. Arts, P.M. James, G. Zhengxian, F.J. Stuart, G. Le, A.W. Mark, J. Virol. 70 (1996) 712–720.
[7] E. Sabini, S. Hazra, M. Konrad, S.K. Burley, A. Lavie, Nucleic Acids Res. 35 (2007) 186–192.
[8] L. Nasens, E. De Clercq, Herpes 8 (2001) 12–16.
[9] S. Wurtzer, S. Compain, H. Benech, A.J. Hance, F. Clavel, J. Virol. 79 (2005) 14815–14821.
[10] A.A. Johnson, A.S. Ray, J. Hanes, et al., J. Biol. Chem. 276 (2001) 40847–40857.
[11] K. Brinkman, J. Smeitink, J. Romijn, P. Reiss, Lancet 354 (1999) 1112–1115.
[12] C.L. Cherry, S.L. Wesselingh, Antimicrob. Agents Chemother. 51 (2003) 1091–1093.
[13] W. Lewis, M. Dalakas, Nat. Med. 1 (1995) 417–422.
[14] K. Parang, L. Wiebe, E.E. Knaus, Curr. Med. Chem. 7 (2000) 995–1039.
[15] S. Sinha, R. Srivastava, E. De Clercq, R.K. Singh, Nucleosides Nucleotides Nucleic Acids 23 (2004) 1815–1824.
[16] S. Sinha, R. Srivastava, B. Prusty, A.C. Das, R.K. Singh, Nucleosides Nucleotides Nucleic Acids 26 (2007) 773–777.
[17] R.K. Singh, D. Rai, D. Yadav, A. Bhargava, J. Balzarini, E. De Clercq, Eur. J. Med. Chem. 45 (2010) 1078–1086.
[18] L. Goldman, J.W. Marsico, J. Med. Chem. 6 (1963) 413–423.
[19] N. Miller, J. Fox, J. Org. Chem. 29 (1964) 1772–1776.
[20] N. Miller, J. Fox, J. Org. Chem. 28 (1963) 936–941 and references therein.
[21] S. Deeks, H. Kessler, J. Eron, et al., Proceedings of the 4th International Workshop on HIV Drug Resistance & Treatment Strategies, Sitges, Spain. Abstract 5, 2000.
[22] J. Chulay, K. Biron, L. Wang, et al., Adv. Exp. Med. Biol. 458 (1999) 129–134.
[23] J.P. Lalezari, J.A. Aberg, L.H. Wang, et al., Antimicrob. Agents Chemother. 46 (2002) 2969–2976.
[24] S.K. Aggarwal, S.R. Gogu, S.R.S. Rangan, K.C. Agrawal, J. Med. Chem. 33 (1990) 1505–1510.
[25] T. Seki, T. Kawaguchi, K. Juni, Pharm. Res. 7 (1990) 948–952.
[26] T. Kawaguchi, K. Ishikawa, T. Seki, K. Juni, J. Pharm. Sci. 79 (1990) 531–533.
[27] M. Kvasnica, Synlett 14 (2007) 2306–2307.
[28] H. Han, R.I. De Vreue, J.K. Rhie, K.-M.Y. Covitz, P.L. Smith, C.-P. Lee, D.-M. Oh, W. Sadee, G.L. Amidon, Pharm. Res. 15 (1998) 1154–1159.
[29] E. De Clercq, A. Hof, I. Rosenberg, T. Sakuma, J. Balzarini, P.C. Maudgal, Nature (London) 323 (1986) 464–467.
[30] K.P. Talaro, A. Talaro, Drugs, microbes, host -- the elements of chemotherapy, in: Foundations in Microbiology, fourth ed. Mc Grow Hill, New York, 2002, pp. 348–379.
[31] E. De Clercq, J. Descamps, G. Verhelst, R.T. Walker, A.S. Jones, P.F. Torrence, D. Shugar, J. Infect. Dis. 141 (1980) 563–574.
[32] E. De Clercq, J. Balzarini, P.F. Torrence, M.P. Mertes, C.L. Schmidt, D. Shugar, P. J. Barr, A.S. Jones, G. Verhelst, R.T. Walker, Mol. Pharmacol. 19 (1981) 321–330.
[33] M. Baba, D. Nakjima, R. Schols, J. Pauwels, J. Balzarini, E. De Clercq, Antiviral. Res. 9 (1988) 335–343.
[34] M. Baba, R. Pauwels, J. Balzarini, J. Arnout, J. Desmyter, E. De Clercq, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 6132–6136 and references therein.
[35] F. Rey, F. Barre-Sinoussi, H. Schmidtmayerova, J.C. Chermann, J. Virol. Methods 16 (1987) 239–249.
[36] R. Pauwels, E. De Clercq, J. Desmyter, J. Balzarini, P. Goubau, P. Herdevijn, H. Vanderhaeghe, M. Vandeputte, J. Virol. Methods 16 (1987) 171–185.
[37] D. Yadav, D. Rai, J. Balzarini, E. De Clercq, R.K. Singh, Nucleic Acids Symp. Ser. (Oxf.) 52 (2008) 263–264.