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Antimycobacterial activities of 5-alkyl (or halo)-3′-substituted pyrimidine nucleoside analogs

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ABSTRACT

Several 5-alkyl (or halo)-3′-azido (amino or halo) analogs of pyrimidine nucleosides have been synthesized and evaluated against *Mycobacterium bovis*, *Mycobacterium tuberculosis* and *Mycobacterium avium*. Among these compounds, 3′-azido-5-ethyl-2′,3′-dideoxyuridine (**3**) was found to have significant antimycobacterial activities against *M. bovis* (MIC_{50} = 1 μg/mL), *M. tuberculosis* (MIC_{50} = 10 μg/mL) and *M. avium* (MIC_{50} = 10 μg/mL).

Tuberculosis (TB), a contagious disease that spreads through aerosols, has reached to pandemic proportions, and is the second leading cause of infectious deaths globally. Approx. 9 million people developed TB and 1.7 millions died from this disease in the year 2009 alone.[1] Although TB is considered as a single disease, it can be caused by several mycobacterial species.[2] *Mycobacterium bovis* and *Mycobacterium tuberculosis* (*Mtb*) pose a significant challenge to the clinical management of TB in immunocompetent as well as immunocompromised people such as HIV infected patients.[3] Infection with HIV suppresses the host's immune system, rendering them more susceptible to TB infection, and/or allowing the reactivation of latent TB infection.[4] Co-infection of TB and HIV allows faster progress and worsening of both diseases.[5] Although HIV is considered to be the single most mortality factor for TB, anyone can get infected with TB and some people are at higher risk such as aboriginals, the elderly, the homeless, the health care professionals, and people living in unhygienic and crowded places.[6]

Bacillus Calmette Guerin (BCG) is an attenuated strain of *M. bovis* that is more than >93% homologous to *Mtb.*[7] In humans, *M. bovis* infections have been reported from 4000–5000 BC. Unfortunately, *M. bovis* infections have re-emerged and are causing TB in humans particularly in HIV positive people.[8] In addition, multidrug resistant (MDR) strains of *M. bovis* that are resistant to up to 11 drugs have been isolated.[9] *M. avium* infections are also one of the most serious complications among patients with AIDS.[10,11] Infections caused by *M. avium* are disseminated rather than restricted to lungs. The first-line anti-TB drugs alone are ineffective against this mycobacteria. The American Thoracic Society (ATS) recommends a three-drug regimen including clarithromycin (CAM) or azithromycin (AZM), rifampin (RFP) or rifabutin (RBT), and ethambutol (EB) for pulmonary MAC disease.[12] Resistance to the macrolides occur at such a rate that single drugs are inadequate.[13] Growing resistance to available TB drugs has emerged as a new threat to the world with new untreatable strains of *M. tuberculosis*, XDR-TB (Extensively drug-resistant TB).[14] Drug resistance in TB arises from spontaneous mutations in mycobacterial DNA, and reversal to drug-sensitivity is not observed.[15] Clinical drug-resistance usually occurs due to insufficient treatment and poor patient adherence of the otherwise treatable TB. The MDR/XDR TB is caused by sequential accumulation of mutations in different genes involved in individual drug-resistance.[15] Subsequent transmission of drug-resistant TB to other people exponentially aggravates the problem. Therefore, new drug development is of paramount importance to respond to this medical emergency, especially the one's targeted to distinct bacterial pathways.

In earlier studies, we noted that a 2′-fluoro derivative of thymidine displays appreciable inhibition of *M. bovis* (MIC_{50} = 50 μg/mL).[16] In our next studies, we observed that a 3′-bromo analog of thymidine, 3′-bromo-2′,3′-dideoxythymidine was a better inhibitor of *Mtb* (H37Ra) (MIC_{50} = 5–10 μg/mL).[17] During our recent work, we found that an ethyl group at the C-5 position of the uracil base also contribute to the antimycobacterial activity where a 3′-bromo derivative of 5-ethyl pyrimidine nucleoside provided very good activity against *M. bovis* and *Mtb* (MIC_{50} = 5 μg/mL). However, none of these compounds were inhibitory against

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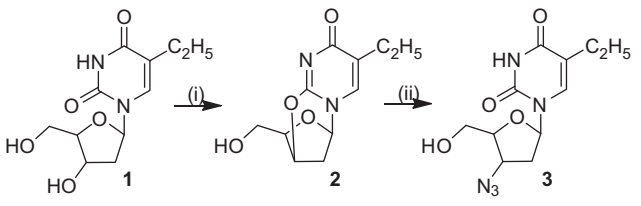
M. avium.[18] In contrast, we observed that 5-ethyl-2′-deoxyuridine was devoid of antimycobacterial activity.[16]

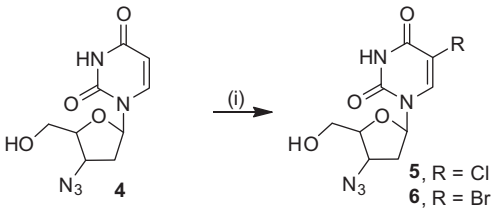
In this article, we have explored various 5-methyl (ethyl or halo)-substituted pyrimidine nucleosides with a variety of 3′-substituents to determine their effect on antimycobacterial activity. Among the compounds investigated, 5-ethyl analog (**3**) of 3′-azido-2′,3′-dideoxyuridine displayed significant inhibition of *M. bovis* (MIC_{50} = 1 μg/mL), *Mtb* (MIC_{50} = 10 μg/mL) and *M. avium* (MIC_{50} = 10 μg/mL) which was equiactive to cycloserine against *M. bovis* and *Mtb*, and more active against *M. avium* than cycloserine.

The nucleoside, 3′-azido-5-ethyl-2′,3′-dideoxyuridine (**3**) was synthesized using a one step process as compared to multi-step synthesis reported by Herdewijn et al.[19] Thus, the reaction of 5-ethyl-2′-deoxyuridine (**1**) with triphenylphosphine and diisopropyl azodicarboxylate in dry CH_3CN, followed by treatment of the obtained 2,3′-anhydro derivative **2** with NaN_3 in dry DMF at 125–130 °C gave the compound **3** in 40% yield (Scheme 1). Chlorination of 3′-azido-2′,3′-dideoxyuridine (**4**) using an efficient procedure with *N*-chlorosuccinimide and NaN_3 in DME-water provided 3′-azido-5-chloro-2′,3′-dideoxyuridine (**5**) in 70% yield in contrast to only 45% yield reported by Aerschot et al.[20] 3′-Azido-5-bromo-2′,3′-dideoxyuridine (**6**) was also obtained in a superior yield (76% Scheme 2) using one step method by *N*-bromosuccinimide in dry DMF as compared to the procedure reported earlier.[21] Lin et al[22] reported the synthesis of 3′-amino-2′,3′-dideoxy-5-methyluridine (**8**) by hydrogenation of 3′-azido-3′-deoxythymidine (**7**) followed by purification of the product using anion-exchange column. We have synthesized **8** by a modified method by the reaction of **7** with triphenylphosphine in dry pyridine followed by the addition of NH_4OH at room temperature. Trituration of the obtained product with ethyl acetate gave pure **8** in 58% yield (Scheme 3).

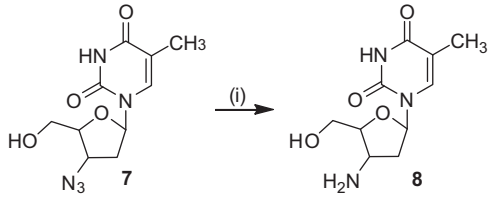
The target nucleosides 3′-iodo-2′,3′-dideoxy-5-ethyluridine (**12**), 2′,3′-dideoxy-5-ethyluridine (**13**), 1-(2-deoxy-β-D-lyxofuranosyl)-5-ethyluracil (**16**), 3′-fluoro-2′,3′-dideoxy-5-trifluoromethyluridine (**19**) and 3′-fluoro-2′,3′-dideoxy-5-ethyluridine (**20**) were synthesized as described in Scheme 4. 5′-O-Tritylation followed by 3′-O-mesylation of the trifluorothymidine (**9**) and 5-ethyl-2′-deoxyuridine (**1**) yielded respective 5′-*O*-trityl-3′-*O*-mesyl derivatives (**10** and **11**) in 79% and 85% yields, respectively. Treatment of **11** with NaI in dry DME followed by detritylation provided **12** in 65% yield. Subsequent hydrogenation of **12** gave deiodinated product **13** in 85% yield. The compounds **12** and **13** were synthesized using reported methods, however, during our reactions, we obtained improved yields of **12** and **13** than reported earlier.[23] Basic hydrolysis of compounds **10** and **11** with NaOH in 90% aqueous ethanol under reflux afforded 1-(5-*O*-trityl-2-deoxy-β-D-lyxofuranosyl)pyrimidines **14** and **15** in 19% and 97% yields, respectively. Detritylation of **15** with 80% aqueous AcOH at 90 °C yielded **16** in 72% yield. In order to prepare 3′-fluoro derivatives **19** and **20**, the compounds **14** and **15** were reacted with (diethylamino)sulfur trifluoride (DAST) in dry THF/dry benzene at room temperature to give 5′-*O*-trityl-3′-fluoro nucleoside derivatives **17** and **18** in 70% and 80% yields, respectively. The compounds



Scheme 1. Reagents and conditions: (i) triphenylphosphine, diisopropyl azodicarboxylate, dry CH_3CN, −15 °C to 0 °C, 4 h, 45% (for **2**); (ii) NaN_3, dry DMF, 125–130 °C, 5 h, 40% (for **3**).



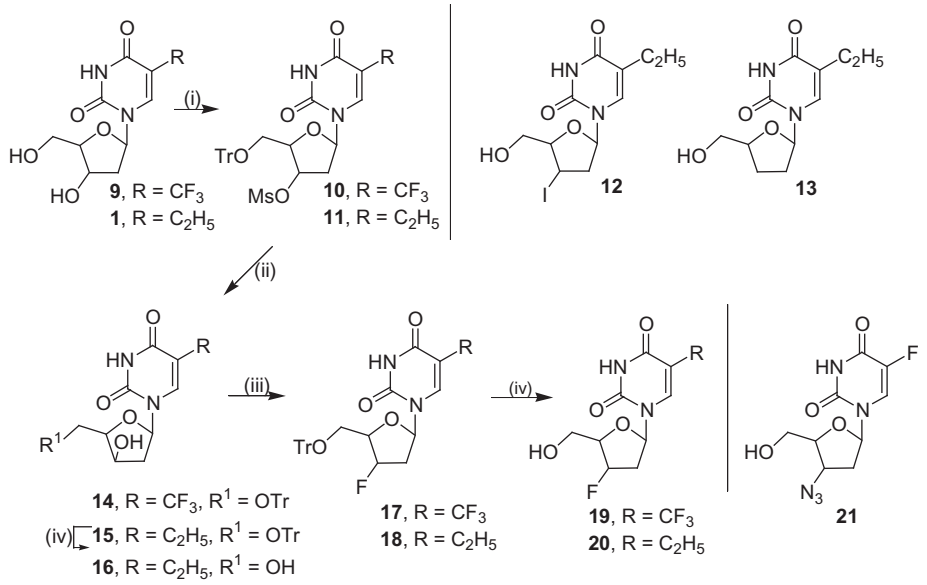
Scheme 2. Reagents and conditions: (i) *N*-chlorosuccinimide, NaN_3, DME–water, room temperature to 45 °C, 70% (for **5**); *N*-bromosuccinimide, dry DMF, room temperature, 76% (for **6**).



Scheme 3. Reagents and conditions: (i) (a) Triphenylphosphine, dry pyridine, room temperature; (b) NH_4OH, room temperature, 58% (for **8**).

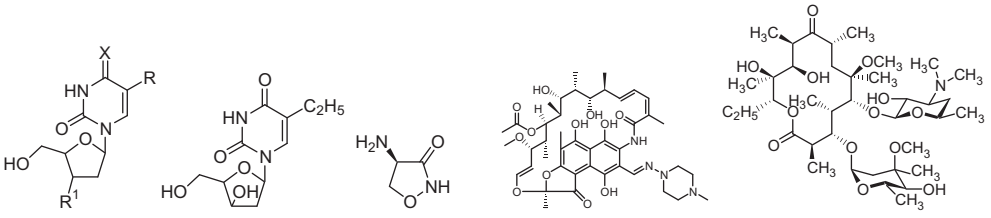
17 and **18**, after detritylation with 80% aqueous AcOH provided 3′-fluoro-2′,3′-dideoxypyrimidine nucleosides **19**[24] and **20** in 46% and 56% yields, respectively. 3′-azido-5-fluoro-2′,3′-dideoxyuridine (**21**) was synthesized using a reported method[25]

The compounds **3, 5, 6, 8, 12, 13, 16, 19–21**, along with reference drugs, cycloserine, rifampicin and clarithromycin were tested against three mycobacteria *M. bovis* (BCG), *M. tuberculosis* (H37Ra) and *M. avium* using microplate alamar blue assay (MABA).[26] The results are summarized in Table 1. Of the compounds tested, 3′-azido-5-ethyl-2′,3′-dideoxyuridine (**3**) was the most active agent against BCG (MIC_{50} = 1 μg/mL). The MIC_{50} exhibited by **3** against BCG was found to be similar to that of cycloserine (MIC_{50} = 1 μg/mL), a second-line anti-TB drug. Encouragingly, *Mtb* strain H37Ra was also inhibited by **3** (MIC_{50} = 10 μg/mL) at 10-times higher concentration than *M. bovis*. Similar pattern of activity against BCG (50% inhibition at 1 μg/mL, MIC_{50} = 1 μg/mL) and *Mtb* H37Ra (60% inhibition at 10 μg/mL, MIC_{50} = 5 μg/mL) was also exhibited by cycloserine. Further, it was interesting to note that **3** possessed activity against *M. avium* (MIC_{50} = 10 μg/mL) also. Although **3** was significantly less active than the reference drug clarithromycin against *M. avium*, it was more potent than cycloserine (15% inhibition at 10 μg/mL). It is noteworthy that the 3′-azido derivative of thymidine (**7**) did not provide inhibition of any mycobacteria in our earlier studies,[16] suggesting that ethyl moiety at the C-5 position in compound **3** contributes to the antimycobacterial activity. Replacement of an azido group at the 3′-position in compound **7** by an amino substituent (**8**) was found to contribute to modest activity against *M. bovis*. In contrast to **3**, the other 3′-azido nucleosides, 3′-azido-5-chloro-2′,3′-dideoxyuridine (**5**) and 3′-azido-5-bromo-2′,3′-dideoxyuridine (**6**) demonstrated lower inhibition of *M. bovis* (50% at 100 μg/mL) and *Mtb* (50% and 30% inhibition, respectively at 100 μg/mL). Whereas, 3′-azido-5-fluoro-2′,3′-dideoxyuridine (**21**) was inactive against all of the mycobacteria tested. These results further indicate that 5-ethyl substituent plays an important role for antimycobacterial activity in this series of compounds. Among other 5-ethyl analogs investigated, only 3′-fluoro derivative (**20**) showed some inhibition of *M. bovis* only at higher concentration (40% inhibition at 100 μg/mL). In contrast, 3′-iodo derivative viz. 3′-iodo-2′,3′-dideoxy-5-ethyluridine (**12**) was devoid of activity which was not surprising since 3′-iodo-2′,3′-dideoxythymidine had not shown activity in our earlier studies.[17] There was no antimycobacterial activity of 3′-deoxy



Scheme 4. Reagents and conditions: (i) Trityl chloride, 4-(dimethylamino)pyridine, dry pyridine, 80 °C, 5–8 h; mesyl chloride, 0 °C, overnight, 79% (for **10**), 85% (for **11**); (ii) NaOH, 90% aq. EtOH, reflux, 1.5–2 h, 19% (for **14**), 97% (for **15**); (iii) DAST, dry benzene, dry THF, room temperature, 2–3 h, 70% (for **17**), 80% (for **18**) (iv) 80% aqueous AcOH, 90 °C, 0.5 h, 65% (for **12**), 72% (for **16**), 46% (for **19**), 56% (for **20**).

Table 1
In vitro antimycobacterial activity of 5-substituted pyrimidine nucleosides against *M. bovis, M. tuberculosis* and *M. avium*



Compd	X	R	R^1	Antimycobacterial activity[a],% inhibition (concentration μg/mL)		
				M. bovis (BCG)	*M. tuberculosis* (H37Ra)	*M. avium* (ATCC 25291)
3	O	C_2H_5	N_3	95% @ 100 and 50 μg/mL, 85% @ 10 μg/mL, 50% @ 1 μg/mL	60% @ 100 and 50 μg/mL, 50% @ 10 μg/mL	100% @ 100 μg/mL, 60% @ 50 μg/mL, 50% @ 10 μg/mL
5	O	Cl	N_3	50% @ 100 μg/mL	50% @ 100 μg/mL	0
6	O	Br	N_3	50% @ 100 μg/mL	30% @ 100 μg/mL	0
8	O	CH_3	NH_2	50% @ 100 μg/mL	0	0
12	O	C_2H_5	I	0	0	0
13	O	C_2H_5	H	0	0	0
16	—	—	—	0	0	0
19	O	CF_3	F	0	0	25% @ 100 μg/mL
20	O	C_2H_5	F	40% @ 100 μg/mL	0	0
21	O	F	N_3	0	0	0
Cycloserine	—	—	—	100% @ 100, 50 and 10 μg/mL, 50% @ 1 μg/mL	100% @ 50 μg/mL, 80% @ 15–20 μg/mL, 60% @ 10 μg/mL, 50% @ 5 μg/mL	100% @ 100 and 50 μg/mL, 15% @ 10 μg/mL
Rifampicin	—	—	—	100% @ 0.5 μg/mL	100% @ 0.5–1 μg/mL	90% @ 2 μg/mL
Clarithromycin	—	—	—	ND[b]	ND	100% @ 2 μg/mL

[a] Antimycobacterial activity was determined at concentrations 100, 50, 25, 10, 5, 1 and 0.5 μg/mL.
[b] ND = not determined.

derivative, 2′,3′-dideoxy-5-ethyluridine (**13**), and the 3′-*lyxo*-hydroxy analog 1-(2-deoxy-β-D-lyxofuranosyl)-5-ethyluracil (**16**) of **3**. These results suggest that both ethyl and azido substituents at the C-5 position of the uracil base and at the 3′-position of the sugar moiety, respectively, are important determinants of anti-TB activity. The 5-trifluoromethyl analog (**19**) of 3′-fluorothymidine showed no antimycobacterial activity in contrast to its 5-ethyl analog **20,** further supporting the role of 5-ethyl group in the antimycobacterial activity.

The XTT and ^{3}H-thymidine incorporation assays were performed to evaluate the toxicity of investigated compounds (**3, 5, 6, 8, 12, 13, 16, 19–21**) in vitro against a human hepatoma cell line (Huh-7). These compounds were not toxic up to the highest concentration tested, 100 μg/mL (CC_{50} >100 μg/mL).

In conclusion, our structure activity relationship studies indicate that both 5-ethyl and 3′-azido substituents together influence the antimycobacterial activity of pyrimidine nucleosides. The 3′-azido-5-ethyl-2′,3′-dideoxyuridine (**3**) that emerged as the most

effective and selective inhibitor, was equiactive to cycloserine against *M. bovis* and *Mtb* H37Ra, and was more potent than cycloserine against *M. avium*. Although compound **3** was significantly less active than a first-line anti-TB drug rifampicin, it could serve as a useful lead compound to improve our understanding of TB drug-design, and develop and optimize therapeutic regimens for TB infections. It is postulated that **3** after metabolic conversion to phosphorylated forms by mycobacterial kinases may be selectively inhibiting *Mtb* DNA and/or RNA synthesis by acting as substrate and/or inhibitor of metabolic enzymes of DNA/RNA synthesis.

In vitro antimycobacterial activity assay: M. bovis (BCG), *M. tuberculosis* (H37Ra), and *M. avium* (ATCC 25291) were obtained from the American Type Culture Collection, Rockville, MD. The antimycobacterial activity was determined using the Microplate alamar blue assay (MABA).[17,26] Test compounds were dissolved in DMSO at 10 mg/mL and subsequent dilutions were performed in 7H9GC (Difco Laboratories, Detroit, Michigan) medium in 96 well plates. For these experiments, each compound was tested at 100, 50, 25, 10, 5, 1, and 0.5 μg/mL concentrations in triplicates. The experiments were repeated two times and the mean percent inhibition is reported in the Table 1. The standard deviations were within 10% of the mean value.

In Vitro Cytotoxicity Assay. Human hepatoma cell line (Huh-7) was used to determine the effect of test compounds on human cell cytotoxicity using XTT and ^{3}H-Thymidine assays. Cell viability was measured using the cell proliferation kit II (XTT; Roche), as per manufacturer's instructions.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.114.

References and notes

1. World Health Organization. Global Tuberculosis Control: Surveillance, Planning, and Financing. WHO Report 2010 WHO Press: Geneva, Switzerland, 2010. Also see the website: http://www.who.int/tb/publications/global_report/2010/en/index.html
2. Addo, K. K.; Owusu-Darko, K.; Yeboah-Manu, D.; Caulley, P.; Minamikawa, M.; Bonsu, F.; Leinhardt, C.; Akpedonu, P.; Ofori-Adjei, D. *Ghana Med. J.* **2007**, *41*, 52.
3. (a) Wells, C. D. *Curr. Infect. Dis. Rep.* **2010**, *12*, 192; (b) Ruíz, P. R.; Esteban, J.; Guerrero, M. L. F. *Clin. Infect. Dis.* **2002**, *35*, 212.
4. Rosas-Taraco, A. G.; Arce-Mendoza, A. Y.; Caballero-Olín, G.; Salinas-Carmona, M. C. *AIDS Res. Human Retroviruses* **2006**, *22*, 45.
5. Mariani, F.; Goletti, D.; Ciaramella, A.; Martino, A.; Colizzi, V.; Fraziano, M. *Curr. Mol. Med.* **2001**, *1*, 209.
6. Tuberculosis Infection Latent Tuberculosis Infection, Fact Sheet 2008, Communicable Disease Control Department, Haliburton, Kawartha, Pine Ridge District Health Unit, Ontario, Canada. Accessed on 23rd September 2011. Available via website www.hkpr.on.ca/uploadedFiles/TBdisease.pdf
7. Imaeda, T. *Int. J. Syst. Bacteriol.* **1985**, *35*, 147.
8. Koehler, C. S. W. *Modern Drug Dis.* **2002**, *5*, 47.
9. Guerrero, A.; Cobo, J.; Fortün, J.; Navas, E.; Quereda, C.; Asensio, A.; Cañón, J.; Blazquez, J.; Gómez-Mampaso, E. *Lancet* **1997**, *350*, 1738.
10. Inderlied, C. B.; Kemper, C. A.; Bermudez, L. E. *Clin. Microbiol. Rev.* **1993**, *6*, 266.
11. (a) Falkinham, J. O., III *Clin. Microbiol. Rev.* **1996**, *9*, 177; (b) Mdluli, K.; Swanson, J.; Fischer, E.; Lee, R. E.; Barry, C. E., III *Mol. Microbiol.* **2002**, *27*, 1223.
12. Griffith, D. E.; Aksamit, T.; Brown-Elliott, B. A.; Catanzaro, A.; Daley, C.; Gordin, F.; Holland, S. M.; Horsburgh, R.; Huitt, G.; Iademarco, M. F.; Iseman, M.; Olivier, K.; Ruoss, S.; von Reyn, C. F.; Wallace, R. J., Jr.; Winthrop, K. *Am. J. Respir. Crit. Care Med.* **2007**, *175*, 367.
13. Heifets, L.; Mor, N.; Vanderkolk, J. *Antimicrob. Agents Chemother.* **1993**, *37*, 2364.
14. (a) XDR-TB-a Global Threat. Editorial. *Lancet* **2006**, *368*, 964.; (b) Wright, A. *Morbidity and Mortality Weekly Report (MMWR)* **2006**, *55*, 301.
15. Zhang, Y.; Yew, W. W. *Int. J. Tubercul. Lung Dis.* **2009**, *13*, 1320.
16. Johar, M.; Manning, T.; Kunimoto, D. Y.; Kumar, R. *Bioorg. Med. Chem.* **2005**, *13*, 6663.
17. Shakya, N.; Srivastav, N. C.; Desroches, N.; Agrawal, B.; Kunimoto, D. Y.; Kumar, R. *J. Med. Chem.* **2010**, *53*, 4130.
18. Srivastav, N. C.; Shakya, N.; Desroches, N.; Agrawal, B.; Kunimoto, D. Y.; Kumar, R., Unpublished results.
19. Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. *J. Med. Chem.* **1987**, *30*, 1270.
20. Aerschot, A. V.; Everaert, D.; Balzarini, J.; Augustyns, K.; Jie, L.; Janssen, G.; Peeters, O.; Blaton, N.; De Ranter, C.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1990**, *33*, 1833.
21. Lin, T.-S.; Chen, M. S.; McLaren, C.; Gao, Y.-S.; Ghazzouli, I.; Prusoff, W. H. *J. Med. Chem.* **1987**, *30*, 440.
22. Lin, T.-S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109.
23. Chu, C. K.; Schinazi, R. F.; Ahn, M. K.; Ullas, G. V.; Gu, Z. P. *J. Med. Chem.* **1989**, *32*, 612.
24. *Experimental synthesis of 3'-Fluoro-2',3'-dideoxy-5-trifluoromethyluridine* (**19**). The compound **17** (0.07 g, 0.13 mmol) was dissolved in 80% aqueous acetic acid (5 mL) and heated at 90 °C for 0.5 h. Solvent was removed in vacuo and the crude product thus obtained was purified on silica gel column using MeOH/$CHCl_3$ (5:95, v/v) as eluent to give **19** (0.018 g, 46%) as solid; mp 198–200 °C. ^{1}H NMR (DMSO-d_6) δ 2.22–2.63 (m, 2H, H-2′), 3.58–3.69 (m, 2H, H-5′), 4.28 (dm, $J_{4',F}$ = 26.86 Hz, 1H, H-4′), 5.22 (m, 1H, 5'-OH), 5.30 (dm, $J_{3',F}$ = 53.10 Hz, 1H, H-3′), 6.14–6.19 (m, 1H, H-1′), 8.76 (s, 1H, H-6), 11.59 (s, 1H, NH). Anal. Calcd for $C_{10}H_{10}F_4N_2O_4$: C, 40.28; H, 3.38; N, 9.39. Found: C, 40.45; H, 3.47; N, 9.17.
25. Lin, T.-S.; Guo, J.-Y.; Schinazi, R. F.; Chu, C. K.; Xiang, J.-N.; Prusoff, W. H. *J. Med. Chem.* **1988**, *31*, 336.
26. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362.