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Studies on acyclic pyrimidines as inhibitors of mycobacteria

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Abstract—In vitro anti-mycobacterial activities of several 5-substituted acyclic pyrimidine nucleosides containing 1-(2-hydroxyethoxy)methyl and 1-[(2-hydroxy-1-(hydroxymethyl) ethoxy)methyl] acyclic moieties are investigated against three mycobacteria viz. *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium avium*, which cause serious infections and mortality in healthy people as well as patients with AIDS. 1-(2-Hydroxyethoxy)methyl-5-(1-azido-2-haloethyl or 1-azidovinyl) analogs (**4**–**7**), 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-decynyluracil (**37**), and 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-dodecynyluracil (**38**) exhibited significant in vitro anti-tubercular activity against these mycobacteria.

1. Introduction

At the present time, nearly two billion people worldwide are infected with the tubercle bacillus, and the prevalence of active tuberculosis (TB) is increasing.[1–3] The latent infection in many of these individuals may reactivate sometime later in life. Tubercle bacillus remains contained in the presence of effective cellular immunity, however, immunocompromised status such as human immunodeficiency virus (HIV) infection, cancer chemotherapy, or use of immunosuppressive drugs in transplantation provides the single most significant factor in reactivation of the latent TB leading to full clinical disease.[1–3]

Chemotherapy with anti-tuberculosis drugs such as streptomycin, *p*-aminosalicylic acid, isoniazid, and rifampicin revolutionized TB therapy in 1970s, and there was a rapid decline in tuberculosis in many developed countries. However, there is now an ever increasing threat of drug-resistant TB appearing as an epidemic in many countries, particularly because no new classes of TB-specific drugs have been developed since the rifampicin.[3]

Two groups of mycobacteria, *M. tuberculosis* and *M. avium*, pose a significant threat of TB in HIV-infected patients and are often responsible for their deaths.[4] Clinical management of *Mycobacterium avium* complex (MAC) infections is very difficult, because many of the first-line anti-tuberculosis drugs are ineffective against it.[5,6] New macrolides, such as clarithromycin and azithromycin, are used for the treatment of MAC, however, fast development of resistance with macrolide therapy poses a significant medical challenge.[7,8] Therefore, intensive research efforts are required to develop new agents for the treatment of TB and multidrug resistant TB (MDR-TB).

Our recent studies showed that novel 5-(C-1 substituted) alkyl side chains at the C-5 position of pyrimidine nucleosides play a crucial role in contributing to their anti-mycobacterial properties.[9] We reported that 5-(1-hydroxyethyl)-(**1a**) and 5-(1-fluoro-2-haloethyl)-(**1b**,**c**) 2′-deoxyuridines possess significant in vitro anti-mycobacterial activity against *M. avium* and *M. bovis*.[9] These studies prompted us to investigate and resynthesize related 5-(1-hydroxy-2-haloethyl)-(**1d**–**f**) 2′-deoxyuridines to evaluate their anti-mycobacterial activity. We also resynthesized the OAc derivatives (**1g**–**i**) of compounds **1d**–**f** to determine if the anti-mycobacterial activity is influenced by the incorporation of the acetyl groups. Unfortunately, compounds **1d**–**i** were found to be non-inhibitory against *M. avium*, *M. bovis* or *M. tuberculosis* up to a concentration of 100 μg/mL (unpublished results, R. Kumar et al).

Keywords: Tuberculosis; Heterocycle; Pyrimidine; Anti-mycobacterial agent.
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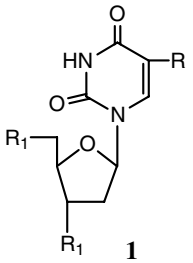


1

1a, R = $CH(OH)CH_3$, R_1 = OH
1b, R = $CH(F)CH_2Br$, R_1 = OAc
1c, R = $CH(F)CH_2Cl$, R_1 = OAc
1d, R = $CH(OH)CH_2Br$, R_1 = OH
1e, R = $CH(OH)CH_2Cl$, R_1 = OH
1f, R = $CH(OH)CH_2I$, R_1 = OH
1g, R = $CH(OH)CH_2Br$, R_1 = OAc
1h, R = $CH(OH)CH_2Cl$, R_1 = OAc
1i, R = $CH(OH)CH_2I$, R_1 = OAc
1j, R = $CH(N_3)CH_2Br$, R_1 = OH
1k, R = $CH(N_3)CH_2Cl$, R_1 = OH
1l, R = $CH(N_3)CH_2I$, R_1 = OH
1m, R = $CH(N_3)=CH_2$, R_1 = OH
1n, R = $C\equiv C\text{-}(CH_2)_7CH_3$, R_1 = OH
1o, R = $C\equiv C\text{-}(CH_2)_9CH_3$, R_1 = OH

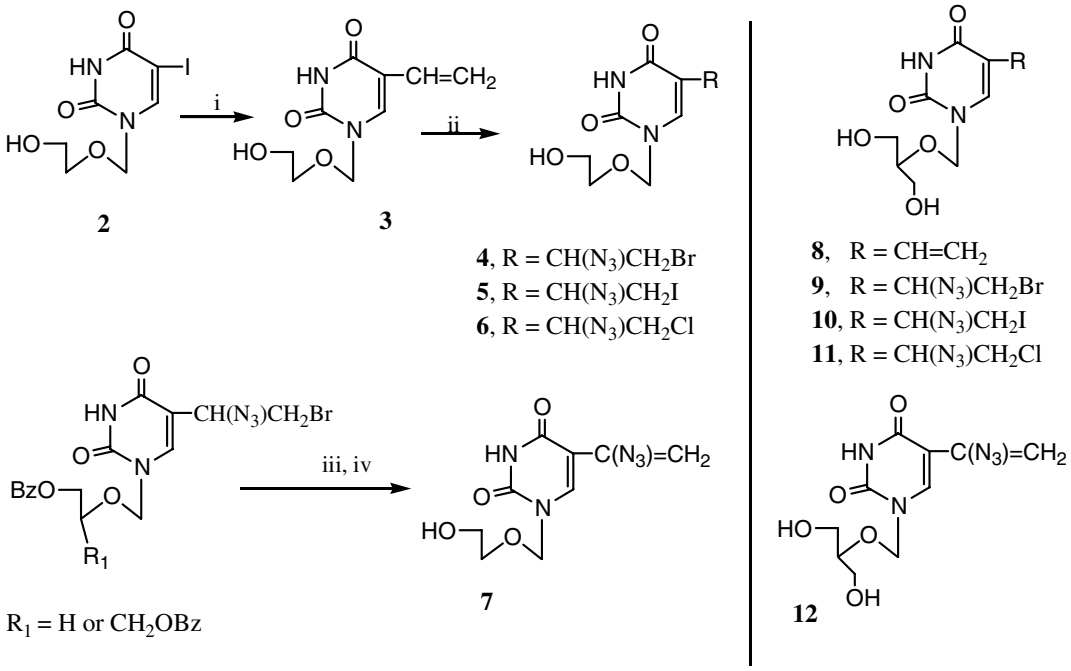
As part of investigation to design more effective anti-mycobacterial agents, an azido moiety located at C-1 position of 5-alkyl side chain of pyrimidine nucleosides attracted our attention as a novel pharmacophore. The azido substituent possesses physicochemical properties relative to other substituents used routinely in structure–activity correlationship studies, such as electronic (inductive effect, *F* value), steric (molecular refractive value), and lipophilic effect (π-value)[10] viz: N_3, 0.30, 10.2, 0.46; F, 0.43, 0.92, 0.14; Cl, 0.41, 6.03, 0.71; Br, 0.44, 8.88, 0.86; I, 0.40, 13.94, 1.12; OH, 0.29, 2.85, −0.67; Me, −0.04, 5.65, 0.56; H, 0.00, 1.03, 0.00, respectively. Thus, the electronic effect of azido is between that of OH and Cl, the steric effect is between that of Br and I, and the lipophilic effect is between that of F and Me. These physical data suggest an azido group may be a good isostere of halogens and OH. In recent studies, we observed that 5-(1-azido-2-haloethyl) analogs of 2′-deoxyuridine (**1j–l**) possessed weak anti-mycobacterial activity against *M. avium*, whereas 5-(1-azidovinyl)-2′-deoxyuridine (**1m**) exhibited significant activity for *M. avium* (MIC_{50} = 1–5 μg/mL).[11] In another study, we reported that 5-alkynyl-2′-deoxyuridines also exhibit notable in vitro anti-mycobacterial activity. We observed that 2′-deoxyuridines with 5-decynyl (**1n**) and 5-dodecynyl (**1o**) terminal alkynes at the C-5 positions were important determinants of anti-mycobacterial activity.[12]

Modified nucleosides have acquired an important role as therapeutic agents in the treatment of patients with devastating cancer and viral infections. One promising class of nucleoside analogs for anti-microbial chemotherapy belongs to a group in which the cyclic carbohydrate moiety is replaced with open-chain 'acyclic' sugar moieties such as 9-(2-hydroxyethoxymethyl)guanine (acyclovir).[13] The effectiveness of acyclic nucleoside analogs as a substrate or inhibitor is likely dependent on the ability of the acyclic side chain to mimic the interaction of the glycosyl portion of the natural substrate with the enzyme. The flexibility of the acyclic chain may allow it to adopt a conformation favorable for enzyme interaction as substrates or inhibitors. Further, it has been demonstrated that acyclic derivatives of pyrimidine nucleosides possess in vivo stabilization against phosphorolysis.[14,15] In contrast, 2′-deoxyuridine analogs can be rapidly catabolized to the corresponding pyrimidine bases by the action of bacterial phosphorylases.[16,17]

In an effort to design novel anti-mycobacterial nucleosides and further explore the structure–activity relationships, in the present communication, we synthesized and evaluated anti-mycobacterial activities of 5-(1-azido-2-haloethyl)-, 5-(1-azidovinyl)-, 5-alkynyl, as well as several other 5-substituted pyrimidine nucleosides in which the furanose moiety is replaced with different acyclic moieties. Interestingly, 1-(2-hydroxyethoxy)methyl-5-(1-azido-2-bromoethyl)uracil (**4**), 1-(2-hydroxyethoxy)methyl-5-(1-azido-2-iodoethyl)uracil (**5**), 1-(2-hydroxyethoxy)methyl-5-(1-azido-2-chloroethyl)uracil (**6**), 1-(2-hydroxyethoxy)methyl-5-(1-azidovinyl)uracil (**7**), 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)-methyl]-5-decynyluracil (**37**), and 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-dodecynyluracil (**38**) were found to be significantly inhibitory to *M. bovis*, *M. tuberculosis*, and *M. avium* replication. To our knowledge, 5-substituted acyclic pyrimidine nucleosides have not previously been reported to possess anti-mycobacterial activity against *M. bovis*, *M. tuberculosis*, and *M. avium*.

2. Chemistry

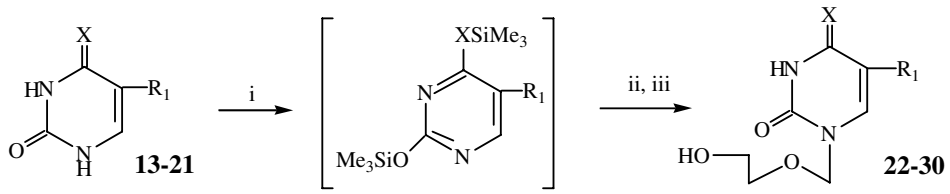
The target compounds, 1-(2-hydroxyethoxy)methyl-5-(1-azido-2-haloethyl) uracils (**4–6**), were synthesized by the reactions of 1-(2-hydroxyethoxy)methyl-5-vinyluracil (**3**) with *N*-bromo(or chloro)succinimide or iodine monochloride, and sodium azide at 25–45 °C using procedures reported by us earlier (Scheme 1).[18] Reaction of benzoylated derivative of **4**, 1-[(2-benzoyloxyethoxy)methyl]-5-(1-azido-2-bromoethyl)uracil, with *t*-BuOK in THF followed by debenzoylation using methanolic ammonia provided the 5-(1-azidovinyl) analog (**7**) (Scheme 1).[19] Similarly, 1-[(2-hydroxy-1-(hydroxymethyl) ethoxy)methyl]-5-(1-azido-2-haloethyl)uracil derivatives (**9–11**) were synthesized by the regiospecific addition of halogenoazides (XN_3: X = Br, Cl, I) to the vinyl substituent of 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-vinyluracil (**8**).[17,18] The reaction of 1-[(2-benzoyloxy-1-(benzoyloxymethyl)ethoxy)methyl]-5-(1-azido-2- bromoethyl) uracil with *t*-BuOK in THF yielded the 5-(1-azidovinyl) compound that was subsequently deprotected using a saturated solution of NH_3 in MeOH to obtain 5-(1-azidovinyl)-analog of 1-[(2-hydroxy-1-(hydroxymethyl) ethoxy)methyl]uracil (**12**).[19]



Scheme 1. Reagents and conditions: (i) $Pd(OAc)_2$, Ph_3P, triethylamine, vinyl acetate, 70 °C; (ii) *N*-bromosuccinimide (**4** and **9**) or *N*-chlorosuccinimide (**6** and **11**), sodium azide, DME–water, 0 °C; iodine monochloride, sodium azide, MeCN (**5** and **10**); (iii) *t*-Bu-OK, THF, 0 °C; (iv) methanolic ammonia, 0–25 °C.

Acyclic pyrimidines **22**–**30** were obtained by a single and convenient route using iodomethyl [(trimethylsilyl)oxy]ethyl ether, prepared in situ from 1,3-dioxolane and trimethyl chlorosilane, instead of acetoxyethyl acetoxymethyl ether as alkylating reagent.[20] Thus, uracils **13**–**21** silylated with bis (trimethylsilyl)acetamide in dry acetonitrile were reacted with trimethylchlorosilane, potassium iodide, and 1,3-dioxolane at room temperature for 16–24 h to yield the desired compounds **22**–**30** in 34–97% yields (Scheme 2).

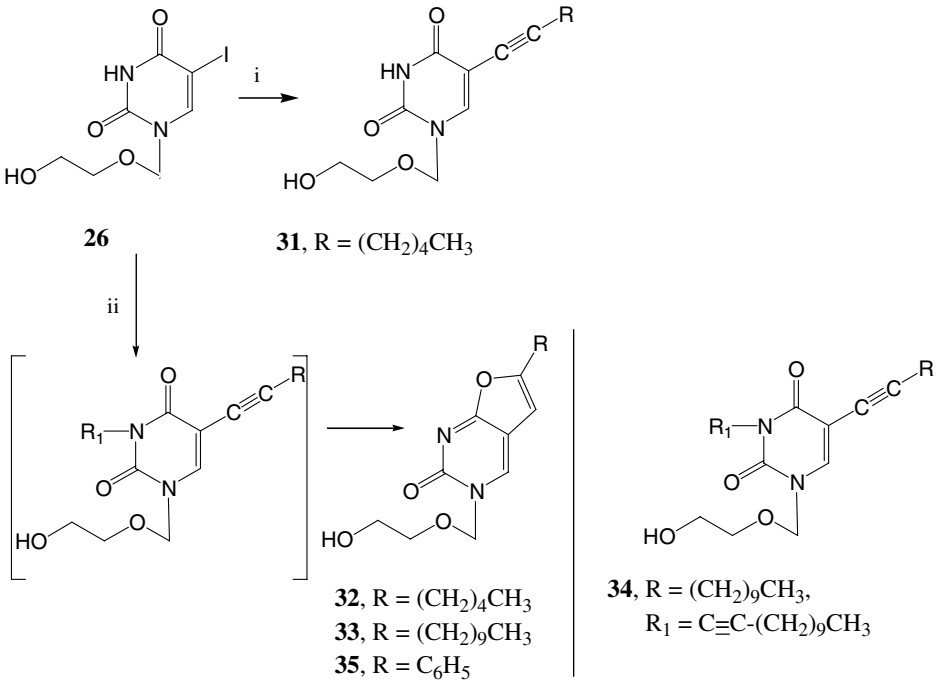
5-Alkynyl acyclic pyrimidine (**31**) was synthesized by the coupling reaction of 5- iodo-1-[(2-hydroxyethoxy)methyl]-uracil (**26**) with 1-heptyne. Bycyclic compounds **32**, **33**, **35** were obtained in one-pot reaction by the Pd-catalyzed coupling reaction of respective terminal alkynes and aryl acetylene with **26**, followed by cyclization of the intermediate 5-alkynyluracils with copper iodide and triethylamine in methanol. Reaction of **26** with 1-dodecyne also produced a di-addition compound, 1-[(2-hydroxyethoxy)methyl]-5,N_3-didodecynyluracil (**34**), unexpectedly. 5-Decynyl-(**37**) and 5-dodecynyl- (**38**) pyrimidine analogs were prepared by the treatment of 5-iodo-1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-uracil (**36**) with 1-decyne and 1-dodecyne terminal alkynes as described for compounds **31** and **32**. Cyclization



13 = R_1 = H, X = O
14 = R_1 = F, X = O
15 = R_1 = Cl, X = O
16 = R_1 = Br, X = O
17 = R_1 = I, X = O
18 = R_1 = CH_3, X = O
19 = R_1 = C_2H_5, X = S
20 = R_1 = NO_2, X = O
21 = R_1 = NH_2, X = O

22 = R_1 = H, X = O
23 = R_1 = F, X = O
24 = R_1 = Cl, X = O
25 = R_1 = Br, X = O
26 = R_1 = I, X = O
27 = R_1 = CH_3, X = O
28 = R_1 = C_2H_5, X = S
29 = R_1 = NO_2, X = O
30 = R_1 = NH_2, X = O

Scheme 2. Reagents and conditions: (i) bis(trimethylsilyl)acetamide, dry acetonitrile; (ii) 1,3-dioxolane, KI, trimethyl chlorosilane, 25 °C; (iii) quenched with MeOH and neutralized with $NaHCO_3$.



Scheme 3. Reagents and conditions: (i) Tetrakis(triphenylphosphine)palladium (0), CuI, (*i*-Pr)$_2$EtN, DMF, 25 °C, 1-heptyne; (ii) tetrakis(triphenylphosphine) palladium (0), CuI, (*i*-Pr)$_2$EtN, DMF, 25 °C, 1-heptyne (**32**), 1-dodecyne (**33** and **34**), phenylacetylene (**35**), triethylamine, dry MeOH, reflux.

of 5-alkynyl uracils (**37** and **38**) in presence of copper iodide and triethylamine in dry methanol at reflux temperature yielded compounds **39** and **40** (Scheme 3).

3. Results and discussion

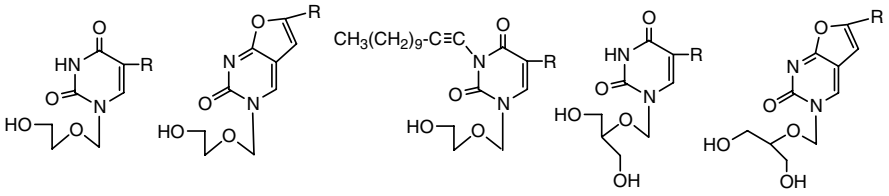
Acyclic pyrimidine nucleosides (**3**–**12** and **22**–**40**) were evaluated in vitro against *M. bovis*, *M. tuberculosis* (H37Ra), and *M. avium* by the microplate Alamar blue assay (MABA) at 1–100 μg/mL concentrations. Rifampicin and clarithromycin were used as reference standards. The results are summarized in Table 1. In these studies, 5-(1-azido-2-haloethyl) derivatives **4**–**6**, with a 1-[(2-hydroxyethoxy)methyl] moiety, emerged as inhibitors of *M. bovis*, *M. tuberculosis*, and *M. avium* where 1-(2-hydroxyethoxy)methyl-5-(1-azido-2-chloroethyl)uracil (**6**) exhibited significant broad spectrum anti-tubercular activity against all three Mycobacteria investigated (MIC_{50} = 10 μg/mL). Interestingly, compounds **4**–**6** were found to exhibit broad spectrum anti-mycobacterial activity in contrast to their corresponding cyclic 2′-deoxyribose analogs (**1j**–**l**) that were only active for *M.avium* (MIC_{50} = 50 μg/mL).[11] Further, a significant shift in the anti-mycobacterial activity of acyclic compound **6** was noted as compared to its 2′-deoxyribose analog (**1k**) which was devoid of anti-mycobacterial activity against all three mycobacteria.[11] Acyclopyrimidines **4**–**6** also showed improved activity in inhibiting the growth of *M. bovis* than 5-(1-halo) substituted analogs, 5-(1-fluoro-2-haloethyl)-2′-deoxyuridines (**1b**,**c**), where 0–25% inhibition was obtained at 50 μg/mL.[9]

Modification of the 5-(1-azido-2-haloethyl) substituent in the acyclic pyrimidine nucleosides **4**–**6** to the corresponding 5-(1-azidovinyl) analog (**7**) provided compound that retained broad spectrum anti-mycobacterial activity similar to compound **6**. In contrast, a 5-vinyl pyrimidine, with 1-[(2-hydroxyethoxy)methyl] substituent (**3**), was not found to be inhibitory to any of the mycobacteria. These results suggest that azido substituent at the C-1 position can be determinant of anti-mycobacterial activity in this series of compounds. In comparison of the anti-mycobacterial activity of acyclic compound **7** with its corresponding 2′-deoxyribose analog (**1m**), we further note that **7** was active against all of the three mycobacteria tested while **1m** was only inhibitory against *M. avium*.[11]

The above results prompted us to study the related 5-(1-azido-2-haloethyl)- (**9**–**11**) and 5-(1-azidovinyl)-(**12**) uracil analogs possessing a different 1-[(2-hydroxy-1-(hydroxymethyl) ethoxy)methyl] acyclic chain in order to determine their anti-mycobacterial activity and identify structure–activity correlations. Surprisingly, compounds **9**–**12** did not inhibit mycobacterial replication, whereas the corresponding 2′-deoxyribose analogs (**1j**–**l** and **1m**) possessed activity against *M. avium* (MIC_{50} = 50 and 1–5 μg/mL, respectively).[11]

Substituents at the 5-position of the pyrimidine nucleosides such as halogens, methyl, ethyl, nitro, and amino have played important role in their biological properties.[21] Based upon these observations, it was of interest to examine the anti-mycobacterial activity of selected acyclic pyrimidine nucleoside analogs (**22**–**30**) containing 1-[(2-hydroxyethoxy)methyl] substituent in our cell-based assays of anti-mycobacterial replication. Interestingly, none of these analogs showed inhibitory effect against *M. bovis*, *M. tuberculosis*, and *M. avium*

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



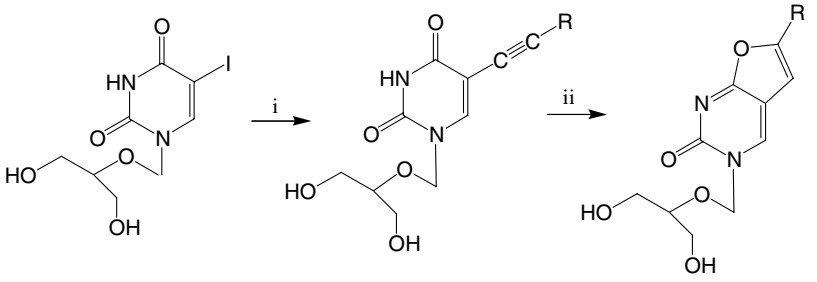
3-7, 22-31 **32,33,35** **34** **8-12, 36-38** **39, 40**

Compound	R	Anti-microbial activity[a] % inhibition (concentration μg/mL)		
		M. bovis (BCG)	*M. tuberculosis* ($H_{37}Ra$)	*M. avium* (ATCC 25291)
3	$CH{=}CH_2$	0	0	0
4	$CH(N_3)CH_2Br$	100 (50)	100 (100, 50), 50 (10)	100 (50)
5	$CH(N_3)CH_2I$	50 (100, 50)	100 (100, 50), 50 (10)	50 (100, 50)
6	$CH(N_3)CH_2Cl$	100 (100, 50) 50 (10)	100 (100, 50), 50 (10)	100 (100, 50) 50 (10)
7	$CH(N_3){=}CH_2$	50 (100, 50, 10)	100 (100, 50), 25 (10)	50 (100, 50, 10)
8	$CH{=}CH_2$	0	0	0
9	$CH(N_3)CH_2Br$	0	0	0
10	$CH(N_3)CH_2I$	0	0	0
11	$CH(N_3)CH_2Cl$	0	0	0
12	$CH(N_3){=}CH_2$	0	0	0
22	H	0	0	0
23	F	0	0	0
24	Cl	0	0	0
25	Br	0	0	0
26	I	0	0	0
27	CH_3	0	0	0
28	C_2H_5	0	0	0
29	NO_2	0	0	0
30	NH_2	50 (100,50)	0	0
31	$CC{\equiv}(CH_2)_4CH_3$	0	0	0
32	$(CH_2)_4CH_3$	50 (100)	25 (100)	0
33	$(CH_2)_9CH_3$	0	0	0
34	$CC{\equiv}(CH_2)_9CH_3$	0	0	0
35	C_6H_5	0	0	0
36	I	0	0	0
37	$CC{\equiv}(CH_2)_7CH_3$	100 (100,50), 60 (10)	100 (100,50), 50 (10)	100 (100), 25 (50)
38	$CC{\equiv}(CH_2)_9CH_3$	100 (100,50), 80 (10), 50 (1)	100 (100,50), 50 (10)	100 (100), 25 (50)
39	$(CH_2)_7CH_3$	90 (100), 50 (50)	75 (100), 50 (50)	50 (100, 50)
40	$(CH_2)_9CH_3$	90 (100, 50), 50 (10)	100 (100), 75 (50), 50 (10)	25 (100)
Rifampicin[b]	—	100 (1)	100 (1)	90 (2)
Clarithromycin[b]	—	ND[c]	ND	95 (2)

[a] Anti-mycobacterial activity was determined at concentrations 100, 50, 10, and 1 μg/mL.
[b] Positive control drugs.
[c] ND, not determined.

up to 100 μg/ml concentration except 1-[(2-hydroxyethoxy)methyl]-5-aminouracil (**30**) that exhibited moderate anti-mycobacterial activity against *M. bovis* (MIC_{50} = 50 μg/mL). These studies suggest that 5-alkyl substituents with an azido group at C-1 position are important for broad spectrum anti-mycobacterial activity (Scheme 4).

Among the 5-alkynyl acyclic pyrimidine nucleosides (**31**, **34**, **37**, **38**), 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)-methyl]-5-decynyluracil (**37**) and 1-[(2-hydroxy-1-(hydroxymethyl) ethoxy)methyl]-5-dodecynyluracil (**38**) provided significant inhibition of all three mycobacteria tested, *M. bovis* (MIC_{50} = 10 and 1 μg/mL, respectively), *M. tuberculosis* (MIC_{50} = 10 μg/mL), and *M. avium* (MIC_{50} = 25 μg/mL). In contrast, 1-[(2-hydroxyethoxy)methyl]-5-heptynyluracil (**31**) and 1-[(2-hydroxyethoxy)methyl]-5,N_3-didodecynyluracil (**34**) were devoid of activity suggesting acyclic moiety, 1-(2-hydroxyethoxy)methyl, present at the N-1 position of **31** and **34** is detrimental to anti-tuberculosis activity. Encouragingly, anti-mycobacterial activity of **37** and **38** against *M. bovis* and *M. avium* was improved as compared to their corresponding 2′-deoxyuridine analogs [**1n**, MIC_{50} = 50 μg/mL (*M. bovis*), MIC_{50} = >100 μg/mL (*M. avium*)], [**1o**, MIC_{50} = >1 μg/mL (*M. bovis*), MIC_{50} = >50 μg/mL (*M. avium*)].[12] This observation was similar to that we noted earlier with acyclic pyrimidines **4**–**6** in contrast to their corresponding 2′-deoxyuridine analogs. Surprisingly, modification of the 5-alkynyl substituent in the acyclopyrimidines (**37** and **38**) to the corresponding bicyclic analogs (**39** and **40**) provided compounds with



36

37, R = $(CH_2)_7CH_3$
38, R = $(CH_2)_9CH_3$

39, R = $(CH_2)_7CH_3$
40, R = $(CH_2)_9CH_3$

Scheme 4. Reagents and conditions: (i) Tetrakis(triphenylphosphine)palladium (0), CuI, $(i\text{-Pr})_2$EtN, DMF, 25 °C, 1-decyne (**37**), 1-dodecyne (**38**); (ii) CuI, triethylamine, dry MeOH, reflux.

similar or slightly diminished anti-mycobacterial activity against all of the three mycobacteria tested, whereas corresponding bicyclic derivatives in the 2′-deoxyuridine series were devoid of any anti-mycobacterial activity.[12] These results suggest that an acyclic glycosyl moiety, 1-(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl, also contributes to the superior anti-mycobacterial activity.

The precise mechanism of action of the active compounds inhibiting mycobacterial multiplication in this study is not clear. The complete genome sequence of *M. tuberculosis* has been deciphered.[22] It encodes many of the enzymes required for DNA and RNA synthesis, and pyrimidine and purine nucleoside biosynthesis. It is postulated that active compounds may be inhibiting mycobacterial DNA and/or RNA synthesis, by acting as substrates and/or inhibitors of metabolic enzymes of DNA and/or RNA synthesis.

The compounds **3**–**12** and **22**–**40** were also evaluated for their anti-bacterial activities against several Gram-positive and Gram-negative (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Enterococcus feacalis*, *Bacillus subtilis*, *Streptococcus pneumoniae*, *Salmonella typhimurium*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) bacteria. However, none of these compounds exhibited any activity at concentrations up to 100 μg/mL suggesting specific action of the most active 5-substituted acyclic pyrimidines (**4**–**7** and **37**–**40**) against mycobacteria. The promising compounds **4**–**7** and **37**–**40** were tested *in vitro* for their toxicity against Vero cells and HepG2 cells up to 100 μg/mL concentration, and no toxicity was observed.

4. Experimental

Melting points were determined with a Buchi capillary apparatus and are uncorrected. ^{1}H NMR spectra were determined for solutions in Me_2SO-d_6 or $CDCl_3$ on a Bruker AM 300 spectrometer using Me_4Si as an internal standard. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of the D_2O. Micro-analyses were within ±0.4% of theoretical values for all elements listed, unless otherwise indicated. Silica gel column chromatography was carried out using Merck 7734 silica gel (100–200 μM particle size). Thin-layer chromatography was performed with Machery-Nagel Alugam SiL G/UV silica gel slides (20 μM thickness).

4.1. 1-[(2-Hydroxyethoxy)methyl]-5-heptynyluracil (31)

To a stirred solution of 1-[(2-hydroxyethoxy)methyl]-5-iodouracil (**26**, 200 mg, 0.64 mmol) in anhydrous dimethylformamide (15 mL), tetrakis(triphenylphosphine)palladium (0) (74.07 mg, 0.064 mmol), copper (I) iodide (24.5 mg, 0.13 mmol), diisopropylethylamine (0.17 mL, 1.0 mmol), and 1-heptyne (0.18 mL, 1.37 mmol) were added. The reaction mixture was stirred at room temperature under nitrogen atmosphere; the progress of the reaction was monitored by TLC in MeOH/EtOAc (1:99, v/v). After 18 h, 15 drops of 5% of disodium salt of EDTA/H_2O were added to the reaction mixture, and the mixture was concentrated in vacuo. The residue obtained was purified on silica gel column using EtOAc as an eluent to yield **31** (50 mg, 28%) as a syrup. ^{1}H NMR (DMSO-d_6) δ 0.97 (t, 3H, J = 6.96 Hz, CH_3), 1.33–1.59 (m, 6H, 3× CH_2), 2.46 (t, 2H, J = 6.96 Hz, α-CH_2), 3.55–3.58 (m, 4H, OCH_2CH_2O), 4.77 (t, J = 5.13 Hz, 1H, OH), 5.18 (s, 2H, NCH_2), 8.10 (s, 1H, H-6), 11.68 (br s, 1H, NH). Anal. Calcd for $C_{14}H_{20}N_2O_4$: C, 60.0; H, 7.14; N, 10.0. Found: C, 60.38; H, 7.0; N, 10.33.

4.2. 3-[(2-Hydroxyethoxy)methyl]-6-pentyl-2,3-dihydro-furo-[2,3-*d*]pyrimidin-2-one (32)

To a stirred solution of 1-[(2-hydroxyethoxy)methyl]-5-iodouracil (**26**, 400 mg, 1.28 mmol) in anhydrous dimethylformamide (25 mL), tetrakis(triphenylphosphine)palladium (0) (150 mg, 0.13 mmol), copper (I) iodide (49 mg, 0.26 mmol), diisopropylethylamine (0.33 mL, 1.89 mmol), and 1-heptyne (0.37 mL, 2.82 mmol) were added. The reaction mixture was stirred at room temperature under nitrogen atmosphere. After 20 h, reaction mixture was cooled down and copper (I) iodide (43 mg, 0.23 mmol), triethylamine (9 mL), and dry methanol (15 mL) were added. The reaction mixture was then heated to reflux and stirred for 4 h. Fifteen drops of 5% of disodium salt of EDTA/H_2O

were added to the reaction mixture, and the mixture was concentrated in vacuo. The residue thus obtained was redissolved in MeOH and filtered to remove the inorganic impurities. The obtained filtrate was concentrated and crude product was purified on silica gel column by using $CHCl_3$/MeOH (96:4) as eluent to give **32** (108 mg, 30%) as a syrup. ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H, J = 6.41 Hz, CH_3), 1.28–1.33 (m, 4H, 2× CH_2), 1.59–1.64 (m, 2H, β-CH_2), 2.64 (t, 2H, J = 7.32 Hz, α-CH_2), 3.45–3.57 (m, 4H, OCH_2CH_2O), 4.68 (t, J = 5.19 Hz, 1H, OH), 5.34 (s, 2H, NCH_2), 6.44 (s, 1H, H-5), 8.45 (s, 1H, H-4). Anal. Calcd for $C_{14}H_{20}N_2O_4$: C, 60.0; H, 7.14; N, 10.0. Found: C, 60.37; H, 7.46; N, 10.29.

4.3. 3-[(2-Hydroxyethoxy)methyl]-6-decyl-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (33) and 1-[(2-hydroxyethoxy)-methyl]-5,N_3-didodecynyluracil (34)

To a stirred solution of 1-[(2-hydroxyethoxy)methyl]-5-iodo-uracil (**26**, 400 mg, 1.28 mmol) in anhydrous dimethylformamide (25 mL), tetrakis(triphenylphosphine)palladium (0) (150 mg, 0.13 mmol), copper (I) iodide (49 mg, 0.26 mmol), diisopropylethylamine (0.33 mL, 1.89 mmol), and 1-dodecyne (0.64 mL, 2.99 mmol) were added. The reaction mixture was stirred at room temperature under nitrogen atmosphere; the progress of the reaction was monitored by TLC in MeOH/$CHCl_3$ (5:95, v/v). After 18 h, reaction mixture was cooled down. To the same reaction mixture, copper (I) iodide (43 mg, 0.23 mmol), triethylamine (9 mL), and dry methanol (15 mL) were added. The reaction mixture was then heated to reflux and stirred for 4 h. Fifteen drops of 5% of disodium salt of EDTA/H_2O were added to the reaction mixture, and the mixture was concentrated in vacuo. The residue thus obtained was redissolved in MeOH and filtered to remove the inorganic impurities. Filtrate was concentrated and crude product was purified on silica gel column by using $CHCl_3$/MeOH as eluent to give compounds **33** and **34** as syrups. Compound **34**, eluent $CHCl_3$/MeOH (98:2); yield (60 mg, 9%); ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 6H, 2× CH_3), 1.22–1.68 (m, 32H, 16× CH_2), 2.26 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 3.45–3.58 (m, 4H, OCH_2CH_2O), 4.66 (m, 1H, OH), 5.38 (s, 2H, NCH_2), 8.55 (s, 1H, H-6). Anal. Calcd for $C_{31}H_{50}N_2O_4$: C, 72.37; H, 9.72; N, 5.44. Found: C, 72.09; H, 9.38; N, 5.8. Compound **33**, eluent $CHCl_3$/MeOH (96:4); yield (120 mg, 27%); ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 3H, CH_3), 1.22–1.32 (m, 14H, 7× CH_2), 1.58–1.64 (m, 2H, β-CH_2), 2.61–2.66 (m, 2H, α-CH_2), 3.44–3.58 (m, 4H, OCH_2CH_2O), 4.66 (m, 1H, OH), 5.35 (s, 2H, NCH_2), 6.42 (s, 1H, H-5), 8.45 (s, 1H, H-4). Anal. Calcd for $C_{19}H_{30}N_2O_4$: C, 65.14; H, 8.57; N, 8.0. Found: C, 65.44; H, 8.82; N, 7.69.

4.4. 3-[(2-Hydroxyethoxy)methyl]-6-phenyl-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (35)

Compound **35** was prepared by using the procedures as described for **33**, except that aryl acetylene was used as terminal alkyne. Yield (80 mg, 22%); ^{1}H NMR (DMSO-d_6) δ 3.47–3.61 (m, 4 H, OCH_2CH_2O), 4.72 (t, J = 5.5 Hz, 1 H, OH), 5.39 (s, 2H, NCH_2), 7.31 (s, 1H, H-5), 7.44–7.53 (m, 3H, aromatic), 7.83–7.85 (m, 2H, aromatic), 8.68 (s, 1H, H-4). Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.89; N, 9.79. Found: C, 63.27; H, 5.27; N, 10.1.

4.5. 1-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-decynyluracil (37)

To a stirred solution of 5-iodo-1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-uracil (**36**, 200 mg, 0.58 mmol) in anhydrous dimethylformamide (20 mL), tetrakis(triphenylphosphine) palladium(0) (67.5 mg, 0.06 mmol), copper (I) iodide (22.27 mg, 0.12 mmol), diisopropyl ethylamine (0.15 mL, 0.86 mmol), and 1-decyne (0.24 mL, 1.33 mmol) were added. The reaction mixture was stirred at room temperature under nitrogen atmosphere; the progress of the reaction was monitored by TLC in MeOH/EtOAc (1:9, v/v). After 18 h, 15 drops of 5% of disodium salt of EDTA/H_2O were added to the reaction mixture, and then the mixture was concentrated in vacuo. The residue obtained was purified on silica gel column using an initial eluent of EtOAc, followed by an eluent of EtOAc/MeOH (98:2, v/v). The appropriate fractions were combined and the solvent was removed in vacuo to yield **37** (60 mg, 29%) as a syrup. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 7.02 Hz, CH_3), 1.21–1.50 (m, 12H, 6× CH_2), 2.35 (t, 2H, J = 7.32 Hz, α-CH_2), 3.29–3.53 (m, 5H, $OCH_2CH(O)CH_2O$), 4.62 (t, J = 5.5 Hz, 2H, 2× OH), 5.16 (s, 2H, NCH_2), 7.96 (s, 1H, H-6), 11.53 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{28}N_2O_5$: C, 61.36; H, 7.95; N, 7.95. Found: C, 61.63; H, 8.19; N, 7.88.

4.6. 1-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-dodecynyluracil (38)

Compound **38** was prepared using the procedures as described for **37**. Yield (74 mg, 33%); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7.02 Hz, CH_3), 1.27–1.58 (m, 16H, 8× CH_2), 2.37 (t, 2H, J = 7.33 Hz, α-CH_2), 3.63–3.82 (m, 5H, $OCH_2CH(O)CH_2O$), 5.29 (br s, 2H, NCH_2), 7.54 (s, 1H, H-6), 9.0–9.50 (br s, 1H, NH). Anal. Calcd for $C_{20}H_{32}N_2O_5$: C, 63.15; H, 8.42; N, 7.36. Found: C, 62.89; H, 8.36; N, 7.76.

4.7. 3-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl-6-octyl-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (39)

To a stirred solution of **37** (200 mg, 0.57 mmol) in methanol/triethylamine (7:3) (30 mL), copper (I) iodide (25 mg, 0.13 mmol) was added at room temperature under a nitrogen atmosphere. The reaction mixture was then heated to reflux and stirred for 3 h. The solvent was removed in vacuo. The solid thus obtained was redissolved in MeOH and filtered to remove the inorganic impurities. Filtrate was concentrated and purification was conducted on two columns, the first using an eluent $CHCl_3$/MeOH (85:15) and the second an eluent of $CHCl_3$/MeOH (9:1). The appropriate fractions were combined and the solvent was removed in vacuo to yield **39** (66 mg, 33%) as a syrup. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 7.33 Hz, CH_3), 1.23–1.64 (m, 12H, 6× CH_2), 2.63 (t, 2H,

J = 7.02 Hz, α-CH_2), 3.36–3.62 (m, 5H, $OCH_2CH(O)$-CH_2O), 4.64 (t, J = 5.19 Hz, 2H, 2× OH), 5.42 (s, 2H, NCH_2), 6.44 (s, 1H, H-5), 8.49 (s, 1H, H-4). Anal. Calcd for $C_{18}H_{28}N_2O_5$: C, 61.36; H, 7.95; N, 7.95. Found: C, 61.0; H, 8.24; N, 8.21.

4.8. 3-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl-6-decyl-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (40)

Compound **40** was prepared by the cyclization of **38** as described above for **39**, except that the refluxing time was 4 h. Yield (35 mg, 29%); ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 7.02 Hz, CH_3), 1.23–1.32 (m, 14H, 7× CH_2), 1.57–1.65 (m, 2H, β-CH_2), 2.63 (t, 2H, J = 7.33 Hz, α-CH_2), 3.36–3.61 (m, 5H, $OCH_2CH(O)$-CH_2O), 4.64 (m, 2H, 2× OH), 5.42 (s, 2H, NCH_2), 6.44 (s, 1H, H-5), 8.49 (s, 1H, H-4). Anal. Calcd for $C_{20}H_{32}N_2O_5$: C, 63.15; H, 8.42; N, 7.36. Found: C, 63.43; H, 8.75; N, 7.59.

4.9. In vitro anti-mycobacterial activity assay (*M. bovis*, *M. tuberculosis*, *M. avium*)

Mycobacterium bovis (BCG), *M. tuberculosis* ($H_{37}Ra$), and *M. avium* (ATCC 25291) were obtained from the American Type Culture Collection, Rockville, MD. The anti-mycobacterial activity was determined using the Microplate Alamar blue assay (MABA). Test compounds were dissolved in DMSO at 100× of the highest final concentration used and subsequent dilutions were performed in 7H9GC (Difco Laboratories, Detroit, Michigan) media in 96-well plates. For these experiments, each compound was tested at 100, 50, 10, and 1 μ/mL in triplicate. The experiments were repeated three times and the mean percent inhibition is reported in the table. The standard deviations were within 10%. Frozen mycobacterial inocula were diluted in medium 7H9GC and added to each well at 2.5×10^5 CFU/mL final concentration. Sixteen control wells consisted of 8 with bacteria alone (B) and 8 with media alone (M). Plates were incubated for an initial 6 days and starting from 6 days of incubation, 20 μl of 10× Alamar blue and 12.5 μl of 20% Tween 80 were added to one M and one B well. Wells were observed for 24–48 h for visual color change from blue to pink and read by spectrophotometer (at excitation 530/525 and emission 590/535) to determine OD values. If the B well became pink by 24 h (indicating growth), reagent was added to the entire plate. If B well remained blue, additional M and B wells were tested daily until bacterial growth could be visualized by color change. After the addition of the reagent to the plate, cultures were incubated for 24 h and plates were observed visually for color change and also read by spectrophotometer. Visual MIC was defined as the lowest concentration of a compound that prevented a color change from blue to pink. Percent inhibition was calculated as (test well-M bkg./B well-M bkg.) × 100. Similar methodology was used for *M. bovis* BCG, *M. tuberculosis*, and *M. avium*. Rifampicin and clarithromycin were used as positive controls. As negative controls, DMSO was added to the B well at concentration similar to that of compound wells, M wells served as negative controls. In most of the experiments, the M wells gave OD of 3000–4000, and the B wells had OD values 60,000–100,000.

4.10. In vitro anti-bacterial activity assay

A total of nine bacterial strains were used for the determination of the in vitro anti-bacterial activity of the studied compounds. The in vitro anti-bacterial activity was studied by determining their minimum inhibitory concentrations (MICs) by means of the broth microdilution method. Briefly, exponentially growing bacteria were diluted in a liquid sterile medium to obtain a final inoculum of 1×10^4 CFU/mL and subsequently cultured with varying dilutions of compounds for 16–20 h. The minimum inhibitory concentrations were defined as the lowest concentration at which bacterial growth was no longer evident.

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