In vitro Anti-Mycobacterial Activities of Various 2'-Deoxyuridine, 2'-Arabinouridine and 2'-Arabinofluoro-2'-deoxyuridine Analogues: Synthesis and Biological Studies

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Abstract. *M. tuberculosis*, *M. bovis* and *M. avium* infections cause the most important mycobacterioses leading to increased mortality in patients with AIDS. Various 5-substituted 2'-deoxyuridines, arabinouridines, arabinocytidines and 2'-arabinofluoro-2'-deoxyuridines were synthesized and evaluated for their *in vitro* inhibitory activity against *M. bovis*, *M. tuberculosis* and *M. avium*. 5-(C-1 Substituted)-2'-deoxyuridine derivatives emerged as potent inhibitors of *M. avium* (MIC_{50} = 1-10 μg/mL range); 5-(1-azidovinyl)-2'-deoxyuridine being the most active (MIC_{50} = 1-5 μg/mL range). The nature of C-5 substituents appeared to be a determinant of anti-mycobacterial activity.

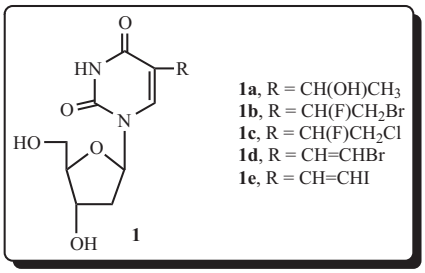
Key Words: Tuberculosis, heterocycle, anti-microbial agent.

INTRODUCTION

One-third of the world's population (~2 billion) is infected with tuberculosis (TB). TB infects eight million people and causes 2-3 million deaths annually around the world [1-3]. Two groups of mycobacteria *M. tuberculosis* and *M. avium* pose a significant challenge to the clinical management of tuberculosis in HIV-infected patients and are often responsible for their death [4]. Bacillus Calmette Guerin (BCG) [5] is an attenuated strain of *M. bovis* that is more than 98% homologous to *M. tuberculosis*. *M. tuberculosis* is most likely an evolved form of *M. bovis*. *M. bovis* infections in humans have been reported from 4000-5000 BC. Interestingly, *M. bovis* infections are back and causing TB in humans particularly those who are HIV positive. In addition, multi-drug resistant (MDR) strains of *M. bovis*, have been isolated [6].

Mycobacterium avium complex (MAC) infections, in particular *M. avium* infections, are one of the most serious complications among patients with acquired immunodeficiency syndrome (AIDS) [7,8]. MAC infections are disseminated rather than restricted to the lungs. Clinical management of MAC infections is very difficult, because many of the first-line anti-TB drugs are ineffective against it [7,8]. New macrolides such as clarithromycin and azithromycin are used for the treatment of MAC, however, resistance occurs at such a rate that single drugs are inadequate for therapy [9,10]. The development of drug-resistant clinical isolates of mycobacteria makes the investigation of new classes of anti-TB agents a high priority.

Our recent studies have shown that novel 5-(C-1 substituted) alkyl side chains at C-5 position of pyrimidine nucleosides play a crucial role in contributing to their antimycobacterial properties [11]. We reported that 5-(1-hydroxyethyl)-(**1a**) and 5-(1-fluoro-2-haloethyl)-(**1b,c**) 2'-deoxyuridines exhibited significant *in vitro* antimycobacterial activity against *M. avium* and *M. bovis*. As part of investigation to design more effective antimycobacterial 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 desirable 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) [12] 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 I, 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 is a good isostere of halogens and OH. Furthermore, the azido group may be capable of electrostatic binding to an enzyme. In our previous studies, we also observed that 5-(1-azido-2-haloethyl) analogs of 2'-deoxyuridine (**3-5**) exhibited biological activities at relatively non-toxic concentrations to host (Vero, HFF) cells [13]. These properties encouraged us to resynthesize pyrimidine nucleosides **3-5** to evaluate them



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1a, R = $CH(OH)CH_3$
1b, R = $CH(F)CH_2Br$
1c, R = $CH(F)CH_2Cl$
1d, R = CH=CHBr
1e, R = CH=CHI

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for their anti-mycobacterial activities. Interestingly, among these compounds, 5-(1-azido-2-bromo(or iodo)ethyl)-2'-deoxyuridines (**3,4**) were found to be moderately inhibitory to *M. avium* replication. These results further prompted us to resynthesize the related 5-(1-azidovinyl)-2'-deoxyuridine analog (**6**) in order to determine its antimycobacterial activity and identify structure activity correlations. Encouragingly, **6** exhibited improved mycobacterial inhibition in contrast to compounds **3**,**4**, with MIC_{50} values of 1-5 μg/ml.

5-Substituted-2'-deoxyuridines can be rapidly catabolized to the corresponding pyrimidine bases by the action of bacterial phosphorylases [14,15]. It has been demonstrated that *in vivo* stabilization of pyrimidine nucleosides against phosphorolysis can be achieved by incorporation of an OH in the arabino configuration of the furanosyl moiety [16]. In an effort to design novel antimycobacterial nucleosides and further explore the structure activity relationships, in the present investigation, we synthesized and evaluated 5-(1-azido-2-haloethyl)-arabinouridines (**10-12**) and 5-(1-azidovinyl)-arabinouridine (**20**) as well as other related analogs of arabinouridine (**21-26**), arabinocytidines (**27,28**) and arabinofluoro-2'-deoxyuridine (**29,30**) for their anti-mycobacterial activity against *M. bovis*, *M. tuberculosis* and *M. avium*.

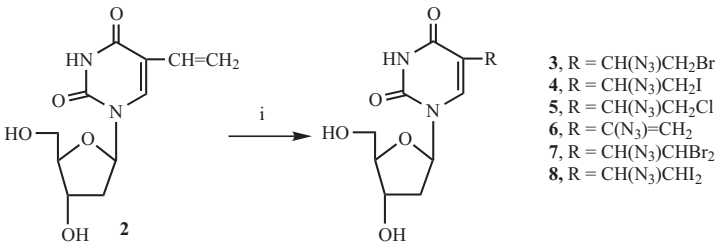
CHEMISTRY

The target 5-(1-azido-2-haloethyl)-2'-deoxyuridines (**3-5**), were synthesized by the reactions of 5-vinyl-2'-deoxyuridine (**2**) with N-bromo(or chloro)succinimide or iodine monochloride, and sodium azide at 25-45 ^{o}C using procedures reported by us earlier [13] (Scheme **1**). Reaction of 3',5'-di-O-acetyl derivative of 5-(1-azido-2-bromoethyl)-2'-deoxyuridine with *t*-BuOK in THF followed by deacetylation gave 5-(1-azidovinyl)-2'-deoxyuridine (**6**) [17]. 5-(1-azido-2,2-dibromo(or chloro)ethyl)-2'-deoxyuridines (**7,8**) were readily prepared by the regiospecific addition of BrN_3 and IN_3 to the vinyl substituent of (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (**1d**) and (*E*)-5-(2-iodovinyl)-2'-deoxyuridine (**1e**), respectively [18]. Similarly, 5-(1-azido-2-haloethyl)-arabinouridines (**10-12**) were synthesized by the regio-specific addition of halogenoazides (XN_3 : X = Br, Cl, I) to the vinyl substituent of 5-vinyl-arabinouridine (**9**) [19]. The reaction of 5-(1-azido-2-bromoethyl)-2',3',5'-tri-O-acetyl-arabinouridine (**13**) with *t*-BuOK in THF did not provide the desired 5-(1-azidovinyl)-2',3',5'-tri-O-acetyl-arabinouridine (**16**) where intramolecular cyclization reaction gave rise to the bicyclic products 5-(2',3',5'-tri-O-acetyl-β-D-arabino-furonosyl)furano[2,3-d] pyrimidin-6-(5H)-one (**14**) and 3-azido-2,3-dihydro-5-(2',3',5'-tri-O-acetyl-b-D-arabinofurono-syl)furano[2,3-d] pyrimidin-6-(5H)-one (**15**) (Scheme **2**). However, a similar reaction with 5-(1-azido-2-bromoethyl)-3',5'-di-O-tert-butyldimethylsilyl analog **18** yielded the desired 5-(1-azidovinyl) product **19** as described in scheme **3**. Deprotection of **19** using n-$Bu4N^{+}F^{-}$ -THF yielded the target 5-(1-azidovinyl)-arabinouridine (**20**) in 58% yield. 5-(1-Cyanamido-2-bromoethyl)-(**21**) and 5-(1-cyanamido-2-iodoethyl)-(**22**) arabinouridines were prepared according our published procedures [20].

The 5-iodo-arabinouridine (**24**) was obtained by the reaction of iodine monochloride and sodium azide at 25-45 ^{o}C using the efficient halogenation procedure reported by us earlier [21]. (*E*)-5-(2-Chlorovinyl)-2'-arabinouridine (**26**) [22] was synthesized from (*E*)-5-(2-carboxyvinyl)-2'-arabinouridine by treatment with N-chlorosuccinimide in N,N-dimethylformamide; that itself was obtained from (*E*)-5-(2-carboethoxyvinyl)-2'-arabinouridine by alkaline hydrolysis [22]. The latter was prepared by reaction of **24** with ethyl acrylate in acetonitrile in the presence of palladium (II) acetate, triphenyl phosphine and triethylamine [22].

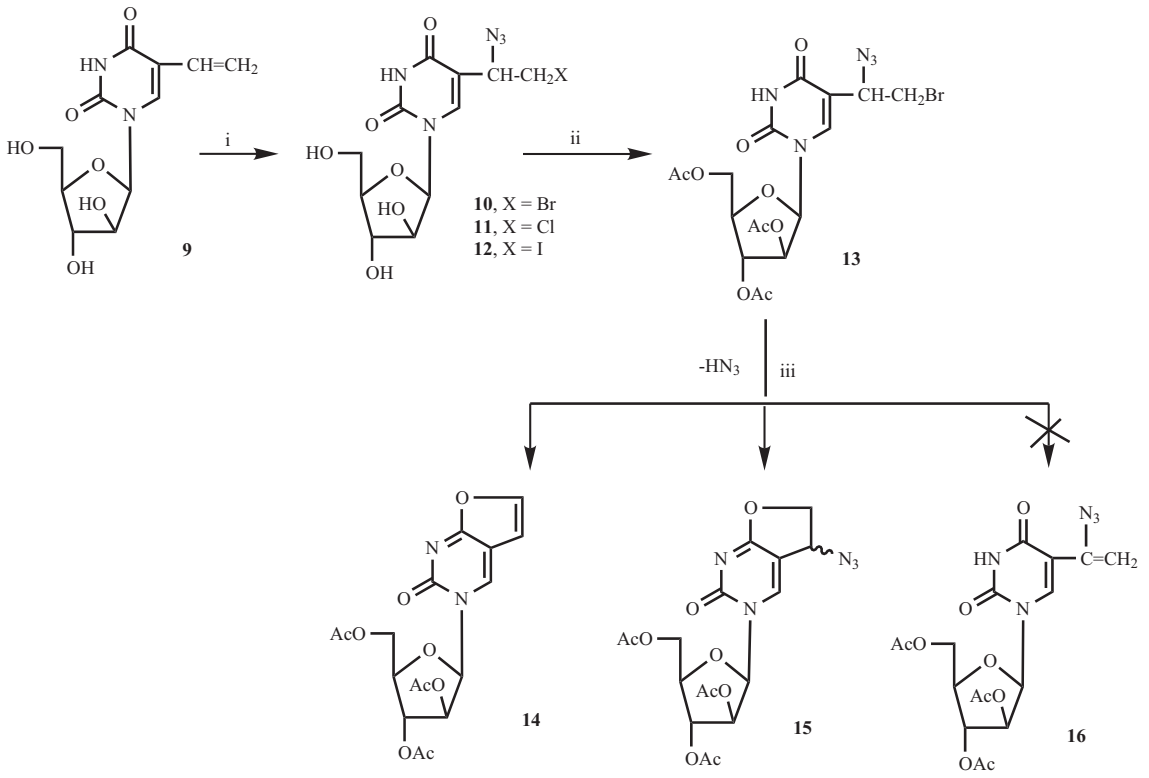
RESULTS AND DISCUSSION:

5-substituted pyrimidine nucleosides (**3-8, 10-12, 20-30**) were evaluated in culture to determine their antimicrobial effect against the multiplication of three mycobacteria (*M. bovis*, *M. tuberculosis*, *M. avium*) using the microplate Alamar blue assay (MABA) [23] at 1-100 μg/mL concentrations. Rifampicin and clarithromycin were used as reference standards. Antimycobacterial activities for these compounds are described in Table **1**. The 5-substituted pyrimidine nucleoside derivatives, modified in the sugar and/or base moiety, evaluated here for their antimycobacterial effect can mainly be divided into three different structural classes: i. 2'-deoxyribose analogs, ii. 2'-arabinose analogs, and iii. 2'-arabinofluoro-2'-deoxyuridine analogs. Among the compounds **3-8, 10-12** and **20-30** tested, 5-(1-azido)- nucleosides possessing 2'-deoxyribose moiety viz., 5-(1-azido-2-bromoethyl)-2'-deoxyuridine (**3**), 5-(1-azido-2-iodoethyl)-2'-deoxyuridine (**4**), and 5-(1-azidovinyl)-2'-deoxyuridine (**6**) were found to exhibit moderate to significant inhibitory activity against *M. avium* (Table **1**). The most potent compound **6** inhibited the growth of *M. avium* with an MIC_{50} of 1-5 μg/mL range. Modification of the 5-(1-



Reagents: i. N-Bromosuccinimide (**3**) or N-chlorosuccinimide (**5**), sodium azide, DME-water, 0 oC; iodine monochloride, sodium azide, MeCN (**4**).

Scheme 1.

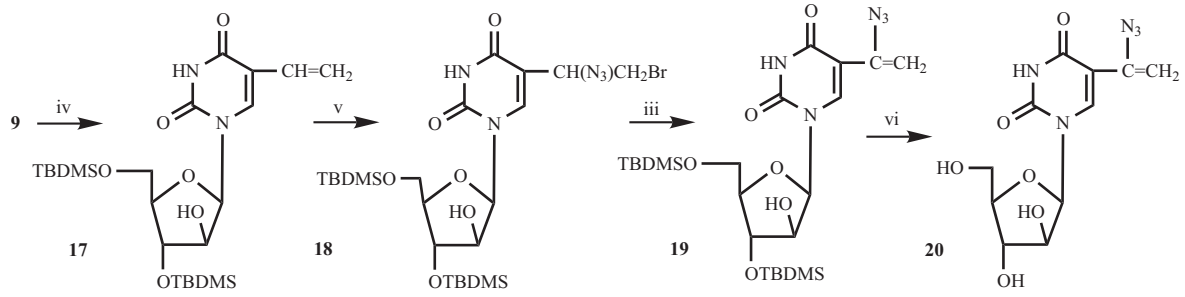


Reagents: i. N-Bromosuccinimide (**10**) or N-chlorosuccinimide (**11**), sodium azide, DME-water, 0 oC; iodine monochloride, sodium azide, MeCN (**12**); ii, acetic anhydride, DMAP, 25 oC; iii. t-BuOK, THF, 0 oC; iv. t-butyldimethylsilyl chloride, imidazole, DMF, 25 oC; v. N-bromosuccinimide, sodium azide, DME-water, 0 oC; vi. n-Bu4N+F-, THF.

Scheme 2.

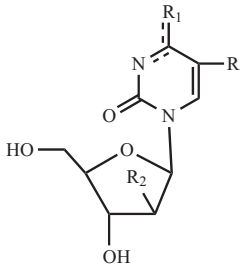
azido-2-haloethyl) substituents in compounds **3** and **4** by inclusion of another halogen atom at the C-2 carbon atom of the 5-substituent was detrimental to anti-mycobacterial activity, as exemplified by 5-(1-azido-2,2-dibromo)-(**7**) and 5-(1-azido-2,2-diiodoethyl)- (**8**) 2'-deoxyuridines that were inactive. Surprisingly, 5-(1-azido-2-haloethyl)-(**10-12**) and 5-(1-azidovinyl)-(**20**) analogs of arabinouridine did not prove to be inhibitory against all of the mycobacteria tested viz. *M. bovis*, *M. tuberculosis* and *M. avium*.

In our earlier studies [20], we observed that a cyanamido (NHCN) group at the C-1 position of the 5-substituent is an efficient bioisostere of both N_3 and OH groups. It was therefore of interest to investigate anti-mycobacterial effect of 5-(1-cyanamido-2-haloethyl)-arabinouridines (**21,22**). Interestingly, compounds **21,22** exhibited notable selective anti-mycobacterial activity against *M. avium* (MIC_{50} = 10 µg/ml) in contrast to their 5-(1-azido) counterparts (**10,11**). Compounds **21,22** exhibited superior anti-mycobacterial



Scheme 3.

Table 1. ***In Vitro*** **Antimycobacterial Activity of Test Compounds Against** ***M. bovis, M. tuberculosis*** **and** ***M. avium***



Compound	R	R_1	R_2	Antimicrobial activity[a] % inhibition (concentration μg/mL)		
				M. bovis (BCG)	*M. tuberculosis* ($H_{37}Ra$)	*M. avium* (ATCC 25291)
3	$CH(N_3)CH_2Br$	OH	H	0	0	50 (50)
4	$CH(N_3)CH_2I$	OH	H	0	0	50 (50)
5	$CH(N_3)CH_2Cl$	OH	H	0	0	0
6	$CH(N_3)=CH_2$	OH	H	0	0	95 (50); 50(1-5)[b]
7	$CH(N_3)CHBr_2$	OH	H	0	0	0
8	$CH(N_3)CHI_2$	OH	H	0	0	0
10	$CH(N_3)CH_2Br$	OH	OH	0	0	0
11	$CH(N_3)CH_2I$	OH	OH	0	0	0
12	$CH(N_3)CH_2Cl$	OH	OH	0	0	0
20	$CH(N_3)=CH_2$	OH	OH	0	0	0
21	$CH(NHCN)CH_2Br$	OH	OH	0	0	75 (50); 50(10)
22	$CH(NHCN)CH_2I$	OH	OH	0	0	75 (50); 50(10)
23	H	OH	OH	0	0	0
24	I	OH	OH	0	0	0
25	CH_3	OH	OH	0	0	0
26	CH=CHCl	OH	OH	0	0	0
27	H	NH_2	OH	0	0	0
28	F	NH_2	OH	0	0	0
29	I	OH	F	0	0	0
30	C_2H_5	OH	F	0	0	0
Rifampicin[c]	-	-	-	100 (1)	100 (1)	90 (2)
Clarithromycin[c]	-	-	-	ND[d]	ND	95 (2)

[a]Antimycobacterial activity was determined at concentrations 100, 50, 10 and 1 μg/mL. [b]Range from three independent repeated experiments. [c]Positive control drugs. [d]ND = Not determined.

activity compared to those of 5-(1-azido-2-bromo (or iodo)ethyl)- (**3,4**) analogs of 2'-deoxyuridine where **3** and **4** showed 50% inhibition at 50 μg/ml.

Substituents at the 5-position of the pyrimidine nucleosides such as halogens, methyl, ethyl and (E)-5-(2-halovinyl) have played important role in their biological properties [22,24]. Based upon these observation, it was of interest to examine the antimycobacterial activity of selected nucleoside analogs viz., 1-β-D-arabinofuranosyluracil (**23**), 1-β-D-arabinofuranosyl-5-iodouracil (**24**), 1-β-D-arabinofuranosylthymine (**25**), (E)-5-(2-chlorovinyl)-arabinouridine (**26**), 1-β-D-arabinofuranosylcytosine (**27**), 1-β-D-arabinofuranosyl-5-fluorouracil (**28**), 5-iodo-2'-arabinofluoro-2'-

deoxyuridine (**29**) and 5-ethyl-2'-arabinofluoro-2'-deoxyuridine (**30**) in cell-based assays. However, none of these arabinouridines, arabinocytidines, and 2'-arabinofluoro-2'-deoxyuridines showed inhibitory effect against *M. bovis, M. tuberculosis* and *M. avium* at 1-100 μg/ml concentrations.

The compounds showing anti-mycobacterial activities were examined for their toxicity *in vitro* in monkey kidney (Vero) cells up to 200 μg/ml concentrations. The compounds **3-6, 21** and **22** did not display toxicity (CC_{50} >200 μg/ml).

METHODS AND MATERIALS

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 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 (TLC) was performed with whatman MK6F silica gel micro slides (25 μM thickness). 1-β-D-arabinofuranosyluracil (**23**), 1-β-D-arabinofuranosylthymine (**25**), 1-β-D-arabinofuranosylcytosine (**27**), and 1-β-D-arabinofuranosyl-5-fluorocytosine (**28**) were purchased from Sigma-Aldrich Chemical Co.

5-(1-Azido-2-bromoethyl)-2',3',5'-tri-O-acetyl-arabinouridine (13)

Acetic anhydride (5 mL) and dimethylaminopyridine (DMAP) (6 mg, 0.05 mmol) were added to a solution of **10** (0.22 g, 0.55 mmol) in dry pyridine (10 mL) at 0-5 °C with stirring. The reaction mixture was allowed to warm to 25 °C, allowed to proceed for 3h and the solvent was removed *in vacuo*. The residue obtained was purified by elution from a silica gel column using chloroform:methanol (97:3, v/v) as eluent to yield **13** (0.24 g, 83.6%) as viscous oil. ^{1}H NMR ($CDCl_3$) : 2.12-2.22 (3s, 9H total, CH_3CO), 3.48-3.92 (m, 2H, CH_2Br), 4.20-4.94 (m, 4H, H-4', H-5', CHN_3), 5.12-5.17 (m, 1H, H-2'), 5.40-5.50 (m, 1H, H-3'), 6.30 and 6.32 (2d, $J_{1',2'}$ = 3.0 Hz, 1H total, H-1'), 7.70 and 7.71 (2s, 1H total, H-6), 8.98 (s, 1H, NH); Anal. for $C_{17}H_{17}N_5O_9Br$: calcd. C, 39.62, H, 3.32, N, 13.59; found. C, 40.93, H, 3.61, N, 13.27.

Reaction of 5-(1-Azido-2-bromoethyl)-2',3',5'-tri-O-acetyl-arabinouridine (13) with Potassium *tert*-butoxide

Potassium *tert*-butoxide (0.1 g, 0.9 mmol) was added to a suspension of **13** (0.22 g, 0.4 mmol) in dry THF (50 mL) at -5 °C with stirring. The cooling bath was removed and the reaction mixture was stirred at 5 °C for 3h. Removal of the solvent *in vacuo* gave a residue which was dissolved in dichloromethane (50 mL), washed with water (10 mL), the dichloromethane fraction was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The reaction mixture was separated by silica gel column chromatography using chloroform-methanol (97:3, v/v) as eluent to give two products as viscous oil which eluted in the following order:

Fraction 1

5-(2',3',5'-tri-O-acetyl-β-D-arabinofuranosyl)furano [2,3-d] pyrimidin-6-(5H)-one (**14**) (52 mg, 32.5%); ^{1}H NMR ($CDCl_3$) : 2.10-2.20 (m, 9H total, CH_3CO), 4.20-4.55 (complex m, 3H, H-4', H-5'), 5.10-5.15 (m, 1H, H-2'), 5.68-5.72 (m, 1H, H-3'), 6.44-6.48 (2d, $J_{1',2'}$ = 3.0 Hz, 1H, H-1'), 6.60 (d, J = 2.5 Hz, 1H, OC*H*=CH), 7.40 (d, J = 2.5 Hz, 1H, OCH=C*H*), 8.36 (s, 1H, H-6); Anal. for $C_{17}H_{18}N_2O_9$: calcd. C, 51.77, H, 4.56, N, 7.10; found. C, 51.47, H, 4.80, N, 7.43.

Fraction 2

3-Azido-2,3-dihydro-5-(2',3',5'-tri-O-acetyl-β-D-arabinofuranosyl)furano [2,3-d]pyrimidin-6-(5H)-one (**15**) (80 mg, 45 %); ^{1}H NMR ($CDCl_3$) : 2.10-2.20 (3s, 9H total, CH_3CO), 4.24-4.68 (complex m, 4H, H-4', H-5', furanyl CHC*H*"), 4.78-4.84 (m, 1H, furanyl CHC*H*"), 5.08 (dd, 1H, furanyl C*H*N_3), 5.16 (m, 1H, H-2'), 5.54-5.60 (m, 1H, H-3'), 6.32 (2d, $J_{1',2'}$ = 3.0 Hz, 1H, H-1'), 8.18 (s, 1H, H-6); Anal. for $C_{17}H_{19}N_5O_9$: calcd. C, 46.68, H, 4.34, N, 16.0; found. C, 46.49, H, 4.18, N, 15.62.

5-Vinyl-3',5'-di-O-tert-butyldimethylsilyl-arabinouridine (17)

Imidazole (0.55 g, 8.0 mmol) and tert-butyl-dimethylsilyl chloride (TBDMSCl) (0.6 g, 4.0 mmol) were added to a solution of **9** (0.5 g, 2.0 mmol) in DMF (20 mL) and the reaction was allowed to proceed at 25 °C with stirring for 48h. Removal of the solvent *in vacuo* and purification of the product by elution from a silica gel column using hexanes-ethylacetate (75:25, v/v) as eluent gave **17** as a viscous oil (0.7 g, 70.0%). ^{1}H NMR ($CDCl_3$) : 0.1-0.22 (m, 12H, $SiMe_2$), 0.93 and 0.97 (2s, 9H each, CMe_3), 3.80-3.86 and 4.0-4.05 (2m, 2H, total, H-5'), 4.06-4.22 (m, 3H, H-2', H-3', H-4'), 5.22 (d, J_{cis} = 11.4 Hz of d, J_{gem} = 2.2 Hz, 1H, CH=C*H*H'), 5.96 (d, J_{trans} = 17.4 Hz of d, J_{gem} = 2.2 Hz, 1H, CH=CH*H*'), 6.12 (d, $J_{1',2'}$ = 3.0 Hz, 1H total, H-1'), 6.36 (d, J_{trans} = 17.4 Hz of d, J_{cis} = 11.4 Hz, 1H, C*H*=CHH'), 7.65 (s, 1H, H-6), 8.28 (s, 1H, NH); Anal. for $C_{23}H_{42}N_2O_6Si_2$: calcd. C, 55.38, H, 8.48, N, 5.61; found. C, 54.94, H, 8.08, N, 5.78.

5-(1-Azido-2-bromoethyl)-3',5'-di-O-tert-butyldimethylsilyl-arabinouridine (18)

N-bromosuccinimide (0.11 g, 0.61 mmol) was added in small aliquots to a precooled suspension at -5 °C, prepared by mixing a solution of **17** (0.3 g, 0.6 mmol) in DME (25 mL) with a solution of sodium azide (0.16 g, 2.5 mmol) in water (250 μL). After the entire 5-vinyl compound **17** had been consumed, the reaction mixture was allowed to stand at 0 °C for 30 min. The solvent was removed *in vacuo* to yield a residue that was purified by elution from a silica gel column using chloroform-methanol (98:2, v/v) as eluent to yield **18** as viscous oil (0.15 g, 31%). ^{1}H NMR ($CDCl_3$) : 0.14, 0.16 and 0.24 (3s, 12H total, $SiMe_2$), 0.93 and 1.0 (2s, 9H each, CMe_3), 3.43-3.56 and 3.57-3.70 (1:1 ratio) (2m, 2H total, CH_2Br), 3.77-4.30 (complex m, 5H, H-2', H-3', H-4' and H-5'), 4.80-4.86 (m, 1H, CHN_3), 6.10 and 6.12 (2d, $J_{1',2'}$ = 3.0 Hz, 1H total, H-1'), 7.71 and 7.73 (2s, 1H total, H-6), 9.30 (s, 1H, NH); Anal. for $C_{23}H_{42}N_5O_6BrSi_2$: calcd. C, 44.50, H, 6.82, N, 11.28; found. C, 44.13, H, 6.67, N, 11.59.

5-(1-Azidovinyl)-3',5'-di-O-tert-butyldimethylsilyl-arabinouridine (19)

Potassium *tert*-butoxide (0.29 g, 2.6 mmol) was added to a suspension of **18** (0.16 g, 0.26 mmol) in dry THF (35 mL) at -5 °C with stirring. The cooling bath was removed and the

reaction mixture was stirred at 5 °C for 2h. Removal of the solvent *in vacuo* gave a residue which was dissolved in chloroform (50 mL) and washed with water (20 mL), the chloroform fraction was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The reaction mixture was separated by silica gel column chromatography using chloroform-methanol (99:1, v/v) as eluent yielded **19** as viscous oil (33 mg, 23%). ^{1}H NMR ($CDCl_3$) δ: 0.1, 0.14 and 0.22 (3s, 12H total, $SiMe_2$), 1.92 and 1.98 (2s, 9H each, CMe_3), 3.81-4.40 (complex m, 5H, H-2', H-3', H-4', H-5'), 5.07 (d, J_{gem} = 2.0 Hz, 1H, C(N_3)=C*H*H'), 6.12 (d, $J_{1',2'}$ = 3.0 Hz, 1H, H-1'), 6.26 (d, J_{gem} = 2.0 Hz, 1H, C(N_3)=CH*H*'), 8.0 (s, 1H, H-6), 8.70 (s, 1H, NH); Anal. for $C_{23}H_{41}N_5O_6Si_2$: calcd. C, 51.17, H, 7.65, N, 12.97; found. C, 51.0, H, 7.63, N, 12.65.

5-(1-Azidovinyl)-arabinouridine (20)

A solution of n-$Bu_4N^+F^-$ (0.15 mL of a 1M solution) in THF was added to a solution of **19** (0.054 g, 0.1 mmol) in THF (10 mL) and the reaction was allowed to proceed with stirring for 6h at 25 °C. Removal of the solvent *in vacuo* and purification of the product on silica gel column using chloroform-methanol (90:10, v/v) as eluent gave **20** (18 mg, 58%). ^{1}H NMR (CD_3OD) δ: 3.86 (m, 2H, H-5'), 3.98 (m, 1H, H-4'), 4.13 (m, 1H, H-3'), 4.22 (m, 1H, H-2'), 5.05 (d, J = 2.0 Hz, 1H, C(N_3)=C*H*H'), 6.09 (d, J = 2.0 Hz, 1H, C(N_3)=CH*H*'), 6.12 (d, J = 3.0 Hz, 1H, H-1'), 8.06 (s, 1H, H-6); Anal. for $C_{11}H_{13}N_5O_6$: calcd. C, 42.44, H, 4.18, N, 22.50; found. C, 42.71, H, 4.45, N, 22.13.

BIOLOGICAL ACTIVITY

In Vitro Antimycobacterial Activity Assay (*M. bovis, M. tuberculosis, M. avium*)

M. bovis (BCG), *M. tuberculosis* (H_{37}Ra) 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) [23]. Test compounds were dissolved in DMSO at 100x 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 micrograms/mL in triplicates. 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 2.5x10^5 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, 20µl of 10x alamar blue and 12.5 µl of 20% Tween 80 were added to one M and one B well. Wells were observed for 24 to 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.) x 100. The lowest drug concentration effecting an inhibition of ~90% was considered as the MIC_{90}. 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 were 60000-100000.

Cell Cytotoxicity Assay

Cell viability was measured using the cell proliferation kit 1 (MTT; Boehringer Mannheim), as per manufacturer's instructions. Briefly, a 96 well plate was seeded with Vero cells or HFF cells at a density of 2.5 x 10^5 cells/well. Cells were allowed to attach for 6-8 h, the media was replaced with media containing drugs at concentrations of 100,50,25,12.5,6.3, and 1.5 µg/mL. DMSO was also included as control. Plates were incubated for 3 days at 37 °C. The color reaction involved adding 10 µl MTT reagent per well, incubating 4 h at 37 °C and then adding 100 µL solubilization reagent. Plates were read on an ELISA plate reader (Abs 560-650 nm) following an overnight incubation at 37 °C.

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