Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6239–6244
Bioorganic & Medicinal Chemistry Letters

Synthesis and antimicrobial activity of some novel nucleoside analogues of adenosine and 1,3-dideazaadenosine

Richa Srivastava,[a,†] Anudita Bhargava[b] and Ramendra K. Singh[a,*]

[a]*Nucleic Acids Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad 211 002, India*
[b]*Head, Microbiology Department, Moti Lal Nehru Medical College, University of Allahabad, Allahabad 211002, India*

Received 16 June 2007; revised 31 August 2007; accepted 5 September 2007
Available online 8 September 2007

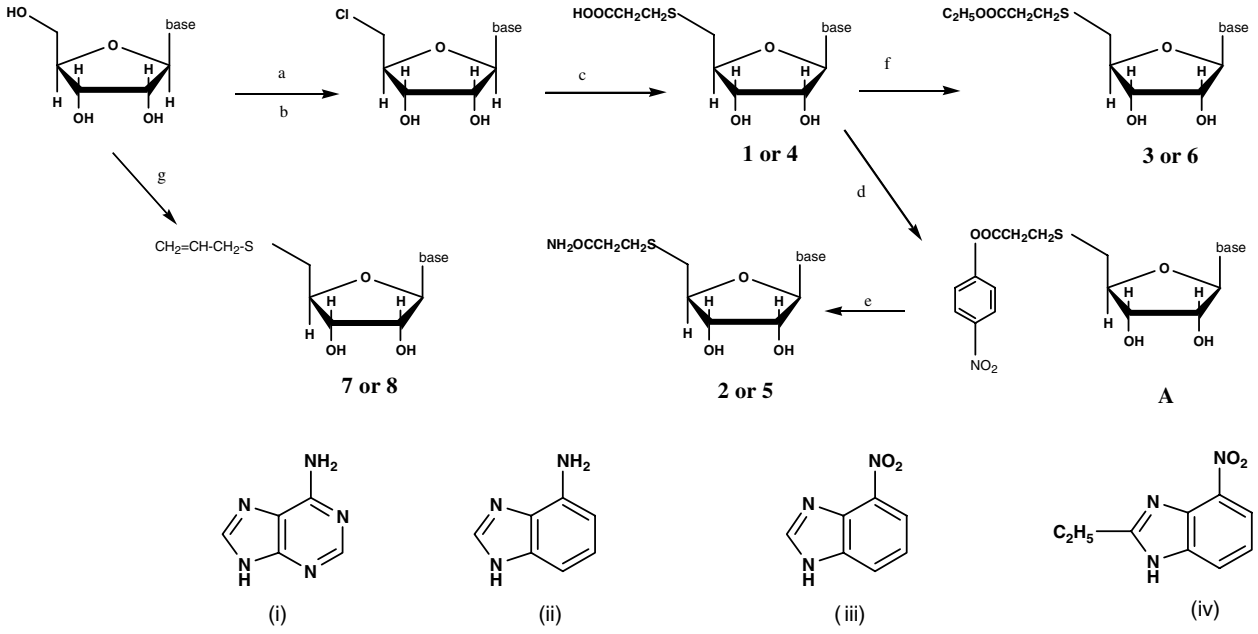
Abstract—A number of nucleoside analogues have been synthesized and evaluated for their antibacterial and antifungal activities against *Staphylococcus aureus*, *Group D Streptococcus*, *Pseudomonas aeruginosa*, *Proteus* spp., *Salmonella* spp., *Aspergillus fumigatus*, *Penicillium marneffei*, *Candida albicans*, *Cryptococcus neoformans*, and *Mucor* spp. The compounds **1**, **4**, and **6** emerged as potent antibacterial agents with MIC values of 0.75, 0.38, and 0.19 μM, respectively, against *group D Streptococcus*. Further, the results suggest that the molecules **4**, **6**, and **7** would be potent antifungal agents as they show substantial degree of inhibition toward the growth of pathogenic fungi with MICs of 0.75, 0.38, and 0.38 μM, respectively.

The increasing prevalence of life-threatening fungal diseases and rapidly growing trend of antimicrobial resistance shown especially by Gram positive bacteria necessitate the development of new and more effective antimicrobial agents. Identification of novel antimicrobial drug with unique modes of action is desirable, since microbes resistant to available antimicrobial agents would unlikely be cross-resistant to these newer drugs.[1] The use of nucleoside analogues can be considered as a novel option as they are expected to act at genomic level, and thereby interfere with transcription or replication processes required for microbial survival. Since there are no alternative pathways in the pathogens for these basic metabolic processes, the nucleoside analogues, by inhibiting these basic pathways, can prove to be effective and better antimicrobial agents. Based on the diverse biological activity and chemical application of benzimidazole derivatives,[2–4] we set out to find a new class of drugs derived from benzimidazoles that may be targeted against major enzymes involved in the fungal and bacterial growth. All the newly synthesized analogues have structural similarity with the naturally occurring nucleoside, *S*-adenosylhomocysteine or SAH, which plays a key role in biological transmethylation reactions. SAH is removed by an enzyme SAH hydrolase,[5] and inhibition of this enzyme, in turn, causes feed back inhibition of biological transmethylation, resulting in the production of uncapped mRNAs and, as a result, less efficient translational process. So, targeting SAH hydrolase is useful in developing antifungal agents.[6–8] SAH is also a substrate for SAH nucleosidase found in bacterial methionine salvage pathway which catalyzes the conversion of methylthioadenosine into methylthioribose and adenine.[9] Targeting SAH nucleosidase may result in new antimicrobial agents and provide an alternative to the problem of resistance faced by most of the existing antibiotics. Further, since SAH nucleosidase is not found in humans, its inhibitors are expected to be non-toxic to human beings.

The compounds used in the present study are synthesized as shown in Scheme 1. For the synthesis of ribosides of 1,3-dideazaadenine (ii), 6-nitro-1,3-dideazaadenine, (iii) and 8-ethyl-1,3-dideazaadenine (iv), silylation of the corresponding substituted bases was performed using hexamethyldisilazane (HMDS) and trichloromethylsilane (TCS) in acetonitrile (CH_3CN) at its reflux point. Excess of HMDS and TCS was removed in vacuo after completion of the reaction. The resultant silyl derivatives were used, without further purification for coupling with suitably protected sugar, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (ABR). The coupling reactions were carried out in the presence of $SnCl_4$ as

Keywords: Adenosine; Dideazaadenosine; SAH analogues; Antifungal; Antibacterial.
* Corresponding author. Tel./fax: +91 532 2461005; e-mail: singhramk@rediffmail.com
† Present address: Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.028



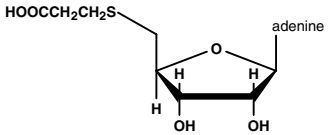
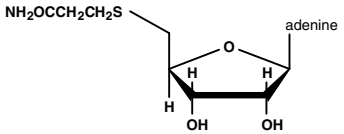
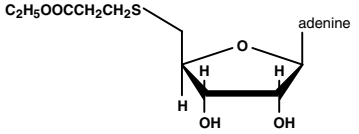
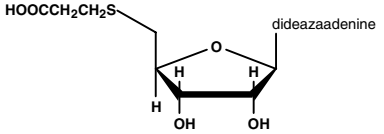
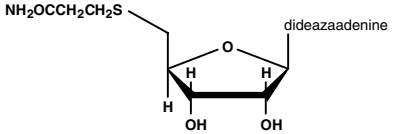
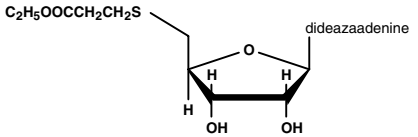
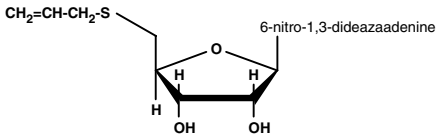
for 1-3, base = (i) adenine
for 4-6, base = (ii) 1,3-dideazaadenine
for 7, base = (iii) 6-nitro-1,3-dideazaadenine
for 8, base = (iv) 6-nitro-8-ethyl-1,3-dideazaadenine

Scheme 1. Reagents: (a) $SOCl_2$/HMPA, dry pyridine; (b) dowex (H^+), 2 N NH_4OH; (c) mercaptopropionic acid, DMAP/pyridine; (d) DCC, *p*-nitrophenol, dioxan; (e) NH_3; (f) DCC, EtOH, concd H_2SO_4; (g) allyl disulfide, triphenyl phosphine.

catalyst using Vorbrüggen and Bennua procedure.[10] The products were purified on silica gel column using DCM/MeOH and deprotected using ammonia (25%) for 5 h at 60 °C. The products were further washed several times with ether and recrystallized using aq ethanol. Adenosine and 9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine were converted to their 5′-chloro-5′-deoxy derivatives by treating them with thionyl chloride ($SOCl_2$) and hexamethylphosphoramide (HMPA).[11] 5′-Chloro-5′-deoxynucleosides were further treated with mercaptopropionic acid to give 5′-*S*-(propionic acid) 5′-deoxy-9-(1′-β-D-ribofuranosyl) adenine (**1**) and 5′-*S*-(propionic acid) 5′-deoxy-9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine (**4**), respectively. The –COOH function of **1** and **4** was activated by a reaction with *p*-nitrophenol and dicyclohexylcarbodiimide (DCC) in pyridine and triethylamine to get the corresponding activated ester **A**. The completion of the reaction was assessed by precipitation of dicyclohexylurea (DCU). The activated phenyl esters were then treated with ammonia to get the corresponding amides—5′-*S*-(propionamide) 5′-deoxy-9-(1′-β-D-ribofuranosyl) adenine (**2**) and 5′-*S*-(propionamide) 5′-deoxy-9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine (**5**). Compounds **1** and **4** when allowed to react with ethanol in the presence of DCC and catalytic amount of concd H_2SO_4 gave the esters-5′-*S*-(ethylpropionate) 5′deoxy-9-(1′-β-D-ribofuranosyl) adenine (**3**) and 5′-*S*-(ethylpropionate) 5′-deoxy-9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine (**6**), respectively. Similarly, glycosylation of 6-nitro-1,3-dideazaadenine (iii) and 8-ethyl-1,3-dideazaadenine (iv) with ABR gave their respective ribosides[12] which were further treated with allyl disulfide and triphenylphosphine to give **7** and **8**. This one step procedure is more convenient than the multistep procedure used earlier for 5′-sulfur generation and the yield is also considerably improved. The advantageous points of this reaction were high yield and absence of side reactions, such as the formation of cyclonucleoside, etc.[13]

All the compounds **1**–**8** were tested in vitro for antibacterial and antifungal activity[14] and they exhibited good to moderate activity against Gram positive bacteria and some human pathogenic fungi, Table 1. The positive results with compounds **1** and **4** against bacteria may be due to easy recognition of propionic acid by the bacterial cells as it is structurally similar to amino acids, the natural components of bacterial cell wall. Thus, the structural features of these molecules help in their better cellular uptake. In order to increase their effectiveness, compounds **1** and **4** were further converted into their derivatives, **3** and **6**, with biodegradable ester linkages. This modification is likely to have made the compounds more lipophilic in nature, which in turn may have made these molecules more effective inhibitors of enzymes involved in lipid and cell wall syntheses. The biodegradable ester linkage gets hydrolyzed inside the cell and the resulting carboxylate form of the drug molecule is thus entrapped inside, resulting in its higher bioavailability. The drug effect was better in its ester form, **6**, than its amide form, **5**, since ester bonds are easily hydrolyzed than amide bonds. The higher activity of these molecules against Gram positive bacteria than Gram negative bacteria might be due to the structural

Table 1. In vitro antibacterial and antifungal activities as MIC (micromole)

Compounds	Structures	*S. aureus*	*S. group D*	*A. fumigatus*	*P. marneffei*	*C. albicans*	*C. neoformans*	*Mucor* spp.
1		nt	0.755	1.5	1.5	1.5	1.5	1.5
2		nt	nt	—	—	—	—	—
3		nt	nt	—	—	—	—	—
4		Nt	0.38	0.75	0.75	0.75	0.75	0.75
5		nt	nt	—	—	—	—	—
6		nt	0.19	0.38	0.38	0.38	0.38	0.38
7		nt	1.25	0.38	0.38	0.38	0.38	0.38

(*continued on next page*)

Table 1 (*continued*)

Compounds	Structures	*S. aureus*	*S. group D*	*A. fumigatus*	*P. marneffei*	*C. albicans*	*C. neoformans*	*Mucor* spp.
8	6-nitro-8-ethyl-1,3-dideazaadenine	nt	nt	2.0	2.0	2.0	2.0	2.0
Vancomycin		15	0.78					
Fluconazole				0.52	0.52	0.52	0.52	0.52
Miconazole				0.77	0.77	0.77	0.77	0.77

nt, not tested, since no clear visible inhibition zone at the disc-diffusion method.
—, resistant.

differences in their cell walls. It was observed that the antimicrobial activity was enhanced when the normal adenine base was replaced by 1,3-dideazaadenine. This enhanced activity establishes the hydrophobic nature of interaction between these compounds and the enzymes involved in microbial proliferation, since it is the bases of nucleosides which are primarily responsible for recognition by enzymes. Further, introduction of 8-ethyl group in 1,3-dideazaadenine, compound **8**, showed significant loss of antimicrobial activity. This clearly shows that any modification at C-8 position impairs the antimicrobial activity of the molecule, probably by proving itself a less favored substrate. Since all the nucleoside analogues are structurally similar to SAH, studies on these molecules on the enzymes SAH hydrolase and SAH nucleosidase (for which SAH is a substrate) as their inhibitor may be helpful in fully evaluating their potential. Further studies are in progress and the detailed work along with mechanism of action of these molecules will be published soon as a full paper separately. Compound **1** has already been shown as a potent anticancer agent against cervical cancer caused by HPV-16 on the basis of studies on HPV-16 +ve SiHa cell line.[15] The synthesis of the molecules studied, their characterization and antimicrobial studies [16] are given under the section references and notes.

Acknowledgments

The authors thank the University Grants Commission, New Delhi, for financial support and the Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow, India, for providing spectral data.

References and notes

1. Projan, S. J. *Curr. Opin. Microbiol.* **2002**, *5*, 513.
2. (a) Canan, K. U. S. *Turk. J. Chem.* **2002**, 559, and references therein; (b) Göker, H.; Ku, C. *Arch. Pharm. (Weinheim)* **1995**, *328*, 425; (c) Garuti, L.; Roberti, M.; Gentilomi, G. *Il Farmaco* **2000**, *55*, 35; (d) Coburn, R. A.; Clark, M. T.; Evans, R. T.; Genco, R. J. *J. Med. Chem.* **1987**, *30*, 205; (e) Roth, T.; Morningstar, M. L.; Boyer, P. L.; Hughes, S. H.; Buckheit, R. W.; Michejda, C. J. *J. Med. Chem.* **1997**, *40*, 4199; (f) Denny, W. A.; Rewcastle, G. W.; Baguley, B. C. *J. Med. Chem.* **1990**, *33*, 814; (g) Evans, D.; Hicks, T. A.; Williamson, W. R. N.; Dawson, W.; Meacock, S. C. R.; Kitchen, E. A. *Eur. J. Med. Chem.* **1996**, *31*, 635.
3. (a) Guckian, K. M.; Morales, J. C.; Kool, E. T. *J. Org. Chem.* **1998**, *63*, 9652; (b) Morales, J. C.; Kool, E. T. *Nat. Struct. Biol.* **1998**, *5*, 950.
4. (a) Gudmundsson, K. S.; Tidwell, J.; Lippa, N.; Koszalka, G. W.; Draanen, N. V.; Ptak, R. G.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **2000**, *43*, 2464; (b) Zou, R.; Ayres, K. R.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1996**, *39*, 3477; (c) Zou, R.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1997**, *40*, 802.
5. (a) Ueland, P. *Pharmacol. Rev.* **1982**, *34*, 223; (b) Palmer, J. L.; Abeles, R. H. *J. Biol. Chem.* **1979**, *254*, 1217.

6. Hasobe, M.; McKee, J. G.; Borchardt, R. T. *Antimicrob. Agents Chemother.* **1989**, *33*, 828.
7. (a) Cools, M.; De Clercq, E. *Biochem. Pharmacol.* **1990**, *40*, 2259; (b) De Clercq, E. *Biochem. Pharmacol.* **1987**, *36*, 2567.
8. Ault-Riche, D. B.; Lee, Y.; Yuan, C.-S.; Hasobe, M.; Wolfe, M. S.; Borcherding, D. R.; Borchardt, R. T. *Mol. Pharmacol.* **1993**, *43*, 989.
9. Tyagi, A. K.; Tabor, C. W.; Tabor, H. *Methods Enzymol.* **1983**, *94*, 294.
10. Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279.
11. Borchardt, R. T.; Huber, J. A.; Wu, Y. S. *J. Org. Chem.* **1976**, *41*, 565.
12. Sinha, S.; Srivastava, R.; Singh, R. K. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 1815.
13. Robins, M. J.; Wnuk, S. F.; Mullah, K. B.; Dalley, N. K.; Yuan, C. S.; Lee, Y.; Borchardt, R. T. *J. Org. Chem.* **1994**, *59*, 544.
14. (a) Baur, A. W.; Kirby, W. M. M.; Shorris, J. C.; Turk, M. *Am. J. Patnol.* **1966**, *45*, 493; (b) Sumana, M. N.; Rajgopal, V. *Ind. J. Pathol. Microbiol.* **2002**, *45*, 169.
15. Sinha S.; Srivastava, R.; Prusty B.; Das, B. C.; Singh, R. K., *Nucleosides, Nucleotides Nucleic Acids* **2007**, in press.
16. Experimental: UV measurements were carried out on a Hitachi 220S spectrophotometer. ^{1}H NMR spectra were recorded using a DRX 300 instrument with DMSO-d_6 as solvent. Fungal strains were obtained from SGPGI, Lucknow, India, and bacterial strains were obtained from clinical patients. *5′-S-(Propionic acid) 5′-deoxy-9-(1′-β-D-ribofuranosyl) adenine (**1**).* 5′-Chloro-5′-deoxyadenosine (10 mmol) and dimethylaminopyridine (DMAP, 0.5 equiv) were dissolved in dry pyridine (25 mL) and stirred for a few minutes to get a clear solution. The solution was made basic by adding triethylamine (TEA, 1.4 equiv or 0.6 mL). Mercaptopropionic acid (12 mmol or 1.2 equiv) in dry pyridine (10 mL) was added to the reaction mixture and the stirring was continued overnight at 50 °C. The reaction mixture was concentrated under vacuum (50–60 °C) to get a dry residue which was then dissolved in water (30 mL). The aqueous solution was washed consecutively with ethyl acetate (15× 3 mL) and was reduced to syrup using a pump. The compound **1** which is contaminated with unreacted mercaptopropionic acid was purified by silica gel column chromatography. Yield 65%, R_f: 0.36 (DCM/MeOH 9.5:05). UV (MeOH) λ_{max} 240, 281, 317 nm. ^{1}H NMR (DMSO-d_6) δ 4.50–4.52 (s, 2H, $NH_{2'}$); δ 8.12 (s, 1H, C-2); δ 8.48–8.70 (s, 1H, C-8); δ 5.65–6.03 (d, J = 6.2, 1H, H1′); δ 3.50–3.70 (m, 1H, H2′); δ 3.49–3.65 (m, 1H, H′3); δ 4.36–4.66 (m, 1H, H4′); δ 3.34–3.46 (m, 2H, H5′); δ 1.90–2.0 (s, 2H, OH); δ 2.50–2.71 (t, 2H); δ 2.55–2.60 (t, 2H); MS *m/z* 354.5 (M^+). Anal. Calcd for $C_{13}H_{17}N_5O_5S$: C, 43.94; H, 4.79; N, 19.72. Found: C, 43.76; H, 4.68; N, 19.57. *5′-S-(Propionamide)5′-deoxy-9-(1′-β-D-ribofuranosyl) adenine (**2**).* Compound **1** (4 mmol) was dissolved in dry pyridine (15 mL) and was stirred for a few minutes to get a clear solution. A solution of *p*-nitrophenol (300 mg) in dioxane (10 mL) was added drop wise to the stirred reaction mixture. After 15 min, TEA (1 mL) was added to make the solution slightly basic. Stirring was continued for another 30 min. DCC (550 mg or 1.2 equiv) dissolved in DCM (10 mL) was added to the reaction vessel. The reaction mixture was stirred overnight at room temperature after adding excess of NH_3. The completion of the reaction is assessed by precipitation of dicyclohexylurea (DCU). The reaction mixture was filtered to separate DCU and the filtrate evaporated to half of its volume. The concentrated solution was poured into 5% $NaHCO_3$ solution (to neutralize the residual acid and to separate excess of PNP) and was extracted with DCM (4× 10 mL). Organic layer was combined, washed with distilled water, and dried over anhydrous Na_2SO_4. The resultant solution was concentrated and the compound **2** crystallized out. The formation of amide was also confirmed by a +ve ninhydrin test for the amino group present at the 5′ position. Yield 45%, R_f = 0.42 (DCM/MeOH 9:1). UV (MeOH) λ_{max} 238, 281, 317 nm. ^{1}H NMR (DMSO-d_6) δ 4.48–4.50 (s, 2H, $NH_{2'}$); δ 8.09 (s, 1H, C-2); δ 8.45–8.68 (s, 1H, C-8); δ 5.68–6.00 (d, J = 6.1, 1H, H1′); δ 3.49–3.69 (m, 1H, H2′); δ 3.46–3.63 (m, 1H, H3′); δ 4.33–4.62 (m, 1H, H4′); δ 3.32–3.40 (m, 2H, H5′); δ 1.88–2.0 (s, 2H, OH); δ 2.70 (t, 2H); δ 2.49 (t, 2H); δ 5.49–6.0 (s, 2H, NH_2); MS *m/z* 353 (M^+). Anal. Calcd for $C_{13}H_{18}N_6O_4S$: C, 44.07; H, 5.08; N, 23.66. Found: C, 43.93; H, 4.98; N, 23.62. *5′-S-(Ethylpropionate) 5′-deoxy-9-(1′-β-D-ribofuranosyl) adenine (**3**).* Compound **1** (4 mmol) dissolved in ethanol (30 mL) was stirred for a few min with concd H_2SO_4 (1.5 mL). To this solution, a solution of DCC (6 mmol) in 10 mL ethanol was added dropwise. Stirring was continued for 24 h at room temperature. The completion of the reaction was assessed by the white ppt of dicyclohexylurea, which was filtered out, and ethanol evaporated on pump at 45 °C. Partitioning between water and ethyl acetate gave the expected product **3** in the organic fraction which was dried over Na_2SO_4, evaporated to a solid mass, and was purified on a silica gel column using a mixture of DCM and MeOH as eluant. Yield 36%, R_f = 0.36 (DCM/MeOH 9:1) UV (MeOH) λ_{max} 257, 281, 317 nm. ^{1}H NMR (DMSO-d_6) δ 4.52–4.54 (s, 2H, $NH_{2'}$); δ 8.10 (s, 1H, C-2); δ 8.45–8.67 (s, 1H, C-8); δ 5.69–6.06 (d, J = 6.1, 1H, H1′); δ 3.54–3.75 (m, 1H, H2′); δ 3.44–3.56 (m, 1H, H3′); δ 4.32–4.59 (m, 1H, H4′); δ 3.33–3.44 (m, 2H, H5′); δ 1.90–2.0 (s, 2H, OH); δ 2.79–2.83 (t, 2H); δ 2.55–2.62 (t, 2H); δ 4.12 (q, 2H); δ 1.30 (t, 3H); MS *m/z* 383.7 (M^+). Anal. Calcd for $C_{15}H_{21}N_5O_5S$: C, 47; H, 5.47; N, 18.23. Found: C, 46.86; H, 5.42; N, 18.19. *5′-Deoxy-9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine (**4**)* **Ribo-DDA** or 9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine was converted to its 5′-chloro-5′-deoxy derivative by treating it with thionyl chloride (1.5 mL) and HMPA (8 mL) which was further converted to **4** by following the procedure and molar ratios similar to that used for the preparation of **1**. TLC indicated the formation of two isomers. One isomer crystallized out as soon as solvent was evaporated on pump while other was still present in mother liquor, which was purified by silica gel column chromatography. The product containing fractions were evaporated and the residue recrystallized from ethanol to give **4**. Yield 45%, R_f = 0.4 (DCM/MeOH, 9.5:0.5). UV (MeOH) λ_{max} 235, 266, 314 nm. ^{1}H NMR (DMSO-d_6) δ 7.26–7.70 (m, 3H, aro); δ 7.90–8.01 (s, 1H, C-8;) δ 8.48–8.70 (s, 1H, C-8); δ 5.65–6.03 (d, 1H, H1′); δ 3.50–3.70 (m, 1H, H2′); δ 3.49–3.65 (m, 1H, H3′); δ 4.36–4.66 (m, 1H, H4′); δ 3.34–3.46 (m, 2H, H5′); δ 1.90–2.0 (s, 2H, OH); δ 2.50–2.71 (t, 2H); δ 2.55–2.60 (t, 2H); MS *m/z* 352.4 (M^+). Anal. Calcd for $C_{15}H_{19}N_3O_5S$: C, 50.99; H, 5.38; N, 11.89. Found: C, 50.81; H, 5.31; N, 11.78. *5′-S-(Propionamide) 5′-deoxy-9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine (**5**)* The compound **5** was obtained from the compound **4** as syrup using a procedure similar to the one used to get compound **2** from **1**. Yield 30%, R_f = 0.4 (DCM/MeOH, 9.5:0.5). UV (MeOH) λ_{max} 235, 266, 314 nm. ^{1}H NMR (DMSO-d_6) δ 7.26–7.70 (m, 3H, aro); δ 7.90–8.01 (s, 1H, C-8); δ 8.48–8.70 (s, 1H, C-8); δ 5.65–6.03 (d, 1H, H1′); δ 3.50–3.70 (m, 1H, H2′); δ 3.49–3.65 (m, 1H, H3′); δ 4.36–4.66 (m, 1H, H4′); δ 3.34–3.46 (m, 2H, H5′); δ 1.90–2.0 (s, 2H, OH); δ 2.72 (t, 2H); δ 2.55 (t, 2H); δ 5.50–6.0 (s, 2H, NH_2); MS *m/z* 353 (M^+). Anal. Calcd for $C_{15}H_{20}N_4O_4S$: C, 51.14; H, 5.68; N, 15.91. Found: C, 51.04; H, 5.55; N,

15.87. *5′-S-(Ethylpropionate) 5′-deoxy-9-(1′-β-D-ribofuranosyl) 1,3-dideazaadenine (**6**)*. Compound **4** was dissolved in ethanol and stirred for few min to get a clear solution and the same procedure was followed as in the case of the analogue **3** to get the compound **6**. Yield 30%, R_f = 0.4 (DCM/MeOH, 9.5:0.5). UV (MeOH) λ_{max} 235, 266, 314 nm. ^{1}H NMR (DMSO-d_6) δ 7.26–7.70 (m, 3H, aro); δ 7.90–8.01 (s, 1H, C-8;) δ 8.48–8.70 (s, 1H, C-8); δ 5.65–6.03 (d, 1H, H1′); δ 3.50–3.70 (m, 1H, H2′); δ 3.49–3.65 (m, 1H, H3′); δ 4.36–4.66 (m, 1H, H4′); δ 3.34–3.46 (m, 2H, H5′); δ 1.90–2.0 (s, 2H, OH); δ 2.79–2.83 (t, 2H); δ 2.55–2.62 (t, 2H); δ 4.12 (q, 2H); δ 1.30 (t, 3H); MS *m/z* 380 (M^+). Anal. Calcd for $C_{17}H_{23}N_3O_5S$: C, 53.54; H, 6.04; N, 11.02. Found: C, 53.47; H, 6.0; N, 10.96. *5′-Allylthio-5′-deoxy-9-(1′-β-D-ribofuranosyl) 6-nitro-1,3-dideazaadenine (7)*. **Ribo-NDDP** or 9-(1′-β-D-ribofuranosyl) 6-nitro-1,3-dideazaadenine[12] (5 mmol) is treated with allyl disulfide (15 mmol) in the presence of Ph_3P (15 mmol) in dry pyridine (50 mL) at room temperature for 24 h. 5′-Allylthio-5′-deoxyribofuranosyl nitrodideazaadenine was obtained as a single product contaminated with unreacted triphenylphosphine. The nucleoside analogue **7** was purified by silica gel column chromatography. Compound **7** eluted between 9 and 11% CH_3OH in CH_2Cl_2. Yield 48%, mp 209 °C, UV (MeOH) λ_{max} 290 nm λ_{min} 238 nm. ^{1}H NMR (DMSO-d_6) δ 7.52–8.19 (m, 4H, aro), 2.3–2.5 (2H, q), 1.3–1.57 (3H, t), 1.8–2.0 (2H, d), 4.0–4.2 (1H, m), 4.8–5.02 (2H, d). MS *m/z* 351.6 (M^+). Anal. Calcd for $C_{15}H_{17}O_5N_3S$: C, 51.28; H, 4.84; N, 11.97. Found: C, 51.21; H, 4.80; N, 11.91. *5′-Allylthio-5′-deoxy-9-(1′-β-D-ribofuranosyl) 8-ethyl-1,3-dideazaadenine (**8**)*. Compound **8** was synthesized by a procedure similar to that described for **7**, from 9-(1′-β-D-ribofuranosyl) 8-ethyl-1,3-dideazaadenine (prepared indigenously).[12] The pure compound **8** was crystallized from ethanol. Yield 58%, mp 189 °C, UV (MeOH) λ_{max} 316 nm, λ_{min} 238 nm. ^{1}H NMR (DMSO-d_6) δ 7.52–8.09 (m, 3H, aro), 2.3–2.5 (2H, q), 1.3–1.57 (3H, t), 1.8–2.0(2H, d), 4.0–4.2 (1H, m), 4.8–5.02 (2H, d). MS *m/z* 332.8 (M^+). Anal. Calcd for $C_{15}H_{17}O_5N_3S$: C, 61.26; H, 6.31; N, 8.41. Found: C, 61.19; H, 6.25; N, 8.37.

Antimicrobial assay: all the nucleoside analogues **1**–**8** were tested in vitro for antibacterial activity against a panel of Gram negative bacilli like *Pseudomonas aeruginosa*, *Proteus* spp., *Salmonella* spp., Gram positive cocci like *Staphylococcus aureus* and *Group D Streptococcus*, and for antifungal activity against human pathogenic fungi like *Aspergillus fumigatus*, *Penicillium marneffei*, *Candida albicans*, *Cryptococcus neoformans*, *Mucor* spp. A known amount of synthesized compounds (1.5; 1.1, 0.57, 0.28, 0.14, and 0.07 μM/mL) was impregnated on sterile filter paper discs of 6mm diameter. Each of the above-mentioned Gram positive and Gram negative bacteria was tested by Kirby Bauer Disc Diffusion (KBDD) method, as per standard protocol (Bauer, et al. *Am. J. Clin. Pathol.* **1966**, *45*, 493). The compounds showing some inhibition zone by this method were further analyzed by the broth-dilution assay to determine their MIC values as per standard protocol (*J. Antimicrob. Chemother*. **1991**, *27*, Suppl. D, 1). Since none of the compounds showed any inhibition against Gram negative bacteria by diffusion method, their MIC values were not determined. The synthesized compounds and reference drugs were dissolved in DMSO–H_2O (50%) and doubling dilutions were subsequently prepared. Equal amount of bacteria was added into each tube to bring the turbidity level to 0.5 Mcfarlands. The tubes were incubated at 37 °C for 18–24 h and observed for any visible turbidity the next day. The MIC was noted as the lowest dilution of the new synthetic compound that inhibited visible growth of the microorganism inoculated. The MIC values are noted in Table 1. Data were not taken for the initial solution because of the high DMSO concentration (12.5%). For antifungal susceptibility testing also the initial screening is done by disc-diffusion method. The broth used is M-3 broth susceptibility test medium and the solid medium used was corn meal agar, instead of Mueller–Hinton agar. Thereafter, the MIC of these nucleoside analogues was evaluated for fungal agents by broth-dilution method (Mazens, M. F. et al. *Antimicrob. Agents Chemother*. **1979**, *15*, 475).